UNIVERSITY of NORTH TEXAS
HEALTH SCIENCE CENTER

FIFTEENTH ANNUAL

RESEARCH Appreciation Day

Center for BioHealth

April 6, 2007

ARCHIVES

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AGENDA

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9:00 - 10:30	Poster Presentation Competition (Preliminary Judging)	Center for BioHealth, 2nd Floor
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11:00 - 12:00	Poster Presentation Competition (Finalist Judging)	Center for BioHealth, 2nd Floor
12:00 - 2:00	Lunch and Keynote Address	Alcon Auditorium, CBH-200

Welcome and Overview of RAD 2007 Activities
Thomas Yorio, Ph.D.
Senior Vice President for Research and
Dean of the Graduate School of Biomedical Sciences

Introduction of Keynote Speaker
Peter Raven, Ph.D.
Professor
Department of Integrative Physiology

"Heart of the Matter: Coronary Dysfunction in Obesity and Insulin"
Johnathan David Tune, Ph.D. (GSBS '97)
Associate Professor, Department of Cellular and Integrative Physiology
Indiana University School of Medicine, Indianapolis, Indiana

2:00 - 5:00	SPH Oral Presentation Competition	Alcon Auditorium, CBH-200
	GSBS Oral Presentation Competition A	Center for BioHealth, 201
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5:00 - 5:30	Remove Posters	Center for BioHealth, 2nd Floor
5:30	Award Ceremony	Alcon Auditorium, CBH-200
6:00 - 8:00	After Party	The GingerMan Pub

(Sponsored by the Graduate Student Association and the Graduate School Alumni Association)

KEYNOTE SPEAKER

Johnathan David Tune, Ph.D. (GSBS '97)

Associate Professor
Department of Cellular and Integrative Physiology
Indiana University School of Medicine
Indianapolis, Indiana

"Heart of the Matter: Coronary Dysfunction in Obesity and Insulin"

Johnathan David Tune, Ph.D. received his doctorate degree at UNT Health Science Center in 1997 under the direction of Fred Downey, Ph.D. in the Department of Integrative Physiology. He went on to perform a postdoctoral fellowship in coronary physiology in the preeminent laboratory of Eric Feigl, M.D. at the University of Washington School of Medicine in Seattle from 1997-2000. After completing his postdoctoral studies, Dr. Tune joined the UNTHSC faculty in 2000 as an Assistant Professor in the Department of Integrative Physiology. In 2003 he accepted an Assistant Professor position in the Department of Physiology at Louisiana State University Health Sciences Center in New Orleans. Following the devastating impact of Hurricane Katrina in 2006, Dr. Tune moved into his current position as Associate Professor of Cellular and Integrative Physiology at the Indiana University School of Medicine in Indianapolis.

Dr. Tune is known for his research on local metabolic and endothelial control of coronary blood flow as well as his recent work to delineate the mechanisms of coronary vascular dysfunction in obesity and insulin resistance. Current studies in his laboratory also focus on the role of adipokines in the pathogenesis of coronary atherosclerotic disease. His research has been funded by the American Heart Association, the American Diabetes Association and currently by the National Institutes of Health. Dr. Tune continues to serve on study sections for the American Heart Association and the National Heart Lung and Blood Institute (NIH) and he is on the editorial board of the journals *Microcirculation* and the *American Journal of Physiology Heart and Circulatory Physiology.* He is an active member of the American Physiological Society, the American Diabetes Association and the Microcirculatory Society.

Dr. Tune and his wife, Mary, have three children, Johnathan (5), Will (3), and a newborn daughter, Caitie.

ALCON RESEARCH, LTD. AWARDS

THE ALCON GROUP

Alcon is the global leader in the research, development, manufacture and marketing of ophthalmic products, including surgical instruments and accessory products, intraocular lenses, prescription drugs and contact lens care solutions.

Founded in Fort Worth, Texas in 1947, the Alcon group now employs approximately 13,500 individuals around the world. Total sales for 2006 exceeded $4.9 billion, with activity in more than 180 markets. One of the cornerstones of Alcon's success is the company's commitment to Research and Development. Located at the company's headquarters in Fort Worth is the 690,000 square-foot William C. Conner Research Center, the largest and most sophisticated eye research center in the world. Over the next five years, Alcon plans to spend at least $3 billion on eye related research and product development in all of its R&D centers, more than any entity outside of the National Eye Institute.

The Alcon Research, Ltd. Awards are given to the top two basic sciences student oral presentations.
In addition, Alcon Research, Ltd. sponsors the Postdoctoral Fellow Poster Competition Award.
All RAD awards are determined by a panel of judges.

GRADUATE STUDENT ASSOCIATION AWARDS

The Graduate Student Association (GSA) promotes the interests and opinions of the graduate student body, sponsors projects and events beneficial to students, and acts as the voice of students on matters of policy and student welfare.

GSA has co-sponsored Research Appreciation Day since its inception. This year, GSA has provided funding for a session of the basic science oral presentation competition as well as the basic science poster presentation competition.

The GSA Poster Presentation Awards are given to the top three student poster presentations in each session in the basic sciences category. Awardees are determined by a panel of judges.

PUBLIC HEALTH STUDENT ASSOCIATION AWARDS

The Public Health Student Association (PHSA) is a student-government organization within the School of Public Health (SPH) that provides students with a forum for promoting collegiality, engaging in service initiatives and voicing student concerns. The purpose of PHSA is to facilitate student-student and student-faculty communication and cohesiveness with respect to the students' academic, research and service experience at the school. The organization advocates on issues pertaining to curriculum revision, research opportunities, student participation, and financial needs. Ultimately, the PHSA will strive to create a strong and enduring foundation for future successors to build upon.

The objectives of the organization are: 1) provide members with resources that will enhance their educational careers; 2) foster communication among students, SPH faculty, staff, and administration; 3) promote research opportunities through collaborative public health approaches to disease prevention and health promotion; and 4) foster a prosperous graduate school experience for its members.

The Public Health Student Association sponsors Research Appreciation Day student awards for the top two oral presentations and the top two poster presentations by a panel of public health judges.

TEXAS COLLEGE OF OSTEOPATHIC MEDICINE AWARDS

The Texas College of Osteopathic Medicine (TCOM) is committed to clinical research excellence by its students and faculty. TCOM educates osteopathic physicians and physician assistants dedicated to careers in health care, teaching and research. By engaging in scholarly pursuits that contribute to further understanding of health and disease, the faculty and students serve the community, the state and the nation.

The Texas College of Osteopathic Medicine Poster Presentation Awards are given to the top two student/resident poster presentations as determined by a panel of judges.

NORTH TEXAS EYE RESEARCH INSTITUTE
OUTSTANDING RESEARCH ACHIEVEMENT AWARD

The North Texas Eye Research Institute was established in 1992 to seek remedies and preventive measures against some of the most prevalent destroyers of sight. Today, its research teams include researchers from many disciplines at UNT Health Science Center, scientists from Alcon Laboratories and practicing ophthalmologists throughout North Texas.

The Institute conducts its battle against blindness on three fronts:

- Basic Research into the cause and treatment of eye diseases, with special emphasis on vision problems relating to aging, vision-related systemic complication of diabetes, and vision disorders disproportionately affecting minority populations with resulting health disparities.
- Clinical Research to measure the response of patients to new therapies.
- Medical Education of clinicians and scientists.

The North Texas Eye Research Institute will recognize one UNTHSC student for the top presentation in the area of eye and vision research during UNTHSC's Research Appreciation Day as determined by a panel of judges. The recipient of the newly established North Texas Eye Research Institute Outstanding Research Achievement Award will be recognized at the Research Appreciation Day 2007 award ceremony and will be able to use the award money to cover travel expenses for presenting the work at a national or international scientific meeting.

PROFESSIONAL AND CONTINUING EDUCATION RESEARCH AWARDS

Professional and Continuing Education (PACE) is pleased to announce the availability of two research awards for outstanding poster presentations at the University of North Texas Health Science Center's Annual Research Appreciation Day. The awards are the PACE Pre-doctoral Research Award and the PACE Post-doctoral Research Award. PACE is the only accredited continuing education provider in the state of Texas for osteopathic as well as allopathic physicians, physician assistants, nurse practitioners, nurses, social workers, nursing faculty and certified health education specialists. PACE registers more than 18,000 healthcare professionals at more than 500 activities each year in 43 states.

Posters will be judged on the following criteria: quality of the research project and relevance of the research project to the field of professional and continuing education.

TECH FORT WORTH INNOVATION AWARD

The TECH Fort Worth Innovation Award is sponsored by TECH Fort Worth. This award is presented to the research poster which depicts the most innovative research. The award-winning poster must present cutting-edge technology which will have a high impact on both the scientific community as well as the general public.

TECH Fort Worth is a public-private partnership of the City of Fort Worth, The University of North Texas Health Science Center, the TCU Neeley School of Business, and the local business community.

TECH Fort Worth is a non-profit business incubator with a mission to facilitate the creation of technology-based jobs in the City of Fort Worth by attracting, incubating, accelerating, and launching successful technology companies that become financially viable and freestanding.

TECH Fort Worth manages a 20,000 sq. ft. facility that offers executive suites, as well as several laboratories at UNTHSC's Center for BioHealth.

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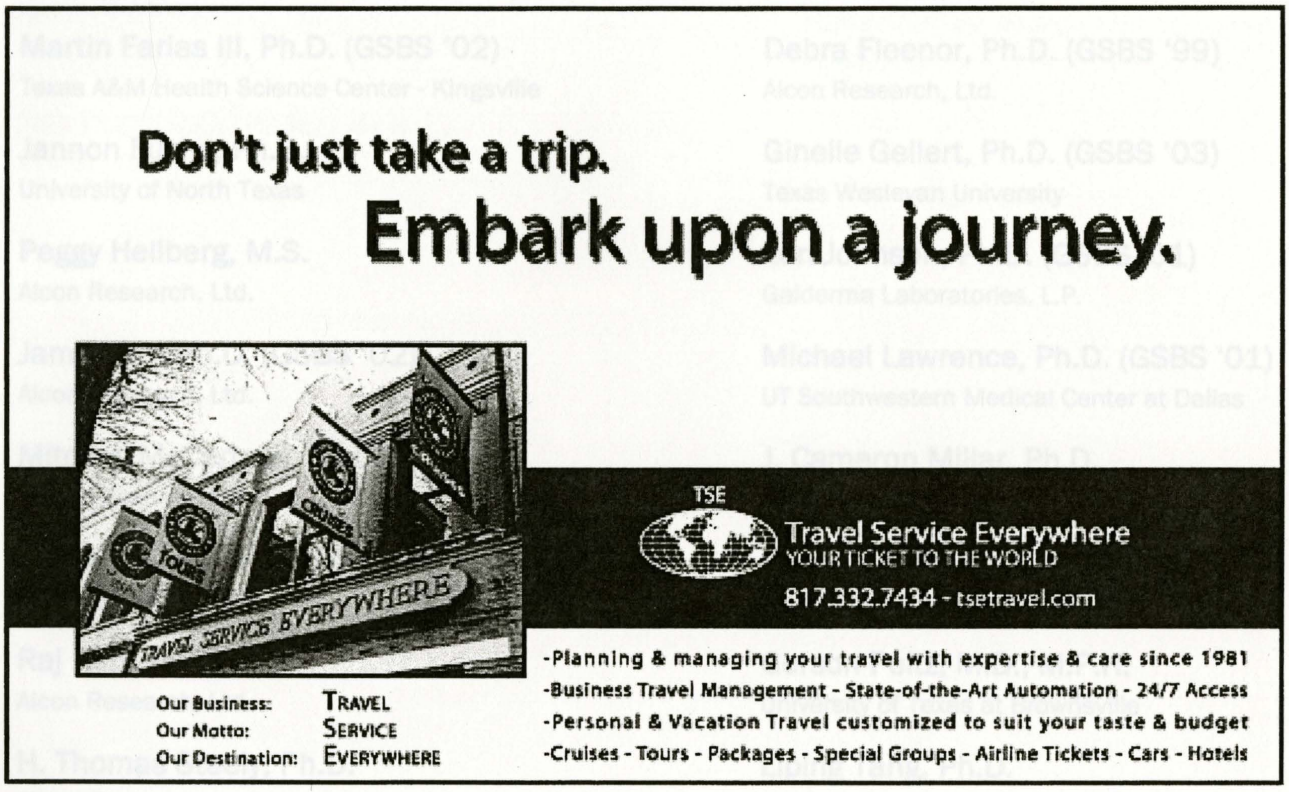
TRAVEL SERVICE EVERYWHERE

Travel Service Everywhere and its affiliates are long-standing supporters of the Graduate School of Biomedical Sciences and UNT Health Science Center. Their support of Research Appreciation Day 2007 includes the donation of one round-trip airline ticket for the first place winner of the basic sciences oral presentation competition to travel to a national scientific meeting.

Please join us in thanking TSE and their fine team of professionals for their continued support of our activities.



JUDGES

Graduate School of Biomedical Sciences judges are:

Bruce Benz, Ph.D.
Texas Wesleyan University

Dennis Cheek, Ph.D., R.N., F.A.H.A.
Texas Christian University

Julie Crider, Ph.D. (GSBS '94)
Alcon Research, Ltd.

Edward Elko, Ph.D.
UNT Health Science Center

Martin Farias III, Ph.D. (GSBS '02)
Texas A&M Health Science Center - Kingsville

Jannon Fuchs, Ph.D.
University of North Texas

Peggy Hellberg, M.S.
Alcon Research, Ltd.

Jami Kern, Ph.D. (GSBS '02)
Alcon Research, Ltd.

Mitchell McCartney, Ph.D.
Alcon Research, Ltd.

Leslie Napier, Ph.D. (GSBS '97)
Alcon Research, Ltd.

Raj Patil, Ph.D.
Alcon Research, Ltd.

H. Thomas Steely, Ph.D.
Alcon Research, Ltd.

Roberta Troy, Ph.D.
Tuskegee University

David Bernard, Ph.D.
University of Texas at Arlington

Abe Clark, Ph.D.
Alcon Research, Ltd.

Oswald D'Auvergne, Ph.D.
Southern University

Eve Ettinger, Ph.D. (GSBS '04)
Alcon Research, Ltd.

Debra Fleenor, Ph.D. (GSBS '99)
Alcon Research, Ltd.

Ginelle Gellert, Ph.D. (GSBS '03)
Texas Wesleyan University

Lori Johnson, Ph.D. (GSBS '01)
Galderma Laboratories, L.P.

Michael Lawrence, Ph.D. (GSBS '01)
UT Southwestern Medical Center at Dallas

J. Cameron Millar, Ph.D.
Alcon Research, Ltd.

Iok-Hou Pang, Ph.D.
Alcon Research, Ltd.

Gerson Peltz, M.D., M.P.H.
University of Texas at Brownsville

Liping Tang, Ph.D.
University of Texas at Arlington

JUDGES

School of Public Health judges are:

Nicole Bereolos, M.P.H. (SPH '04)
UNT Health Science Center

David Crane, M.A., M.P.H. (SPH '03)
UNT Health

Witold Migala, Ph.D. (GSBS '97), M.P.H. (GSBS '95)
Fort Worth Health Department

Texas College of Osteopathic Medicine judges are:

Sharon Clark, D.O., M.P.H.
Burlington Northern

Robert Miley, M.D.
Private Practice

Ray Page, D.O., Ph.D. ('91)
Private Practice

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100 *(Poster)*

Author: Ritu Shetty
Presenter: Nathalie Sumien
Department: Pharmacology & Neuroscience
Classification: Faculty

Ritu A. Shetty, Michael J. Forster, and Nathalie Sumien. Dept of Pharmacology and Neuroscience, UNTHSC, Fort Worth, Texas 76107

CONCURRENT SUPPLEMENTATION OF ALPHA-TOCOPHERYL ACETATE AND COENZYME Q10 DECREASED OXIDATIVE DAMAGE IN BRAIN OF AGED MICE

Purpose: This study was designed to determine whether short-term supplementation with a combination of antioxidants, namely a-tocopheryl acetate and coenzyme Q10 (CoQ10), was more effective than supplementation with either one alone in decreasing oxidative damage in various brain regions of aged mice.

Methods: When 20 months of age, separate groups of mice were assigned to one of the following treatment groups: vehicle (436 mg/kg/d gamma-cyclodextrin (gamma-CD)), TOC (250mg/kg/d alpha-tocopheryl acetate + vehicle), CoQ (109 mg/kg/d CoQ10 + vehicle) or TOC+CoQ (250mg/kg/d alpha-tocopheryl acetate + 109 mg/kg/d CoQ10 + vehicle). A group of 4-month-old mice was used as a young control group receiving the vehicle. The mice in these groups were gavaged daily with their respective treatments for a period of three weeks after which they were euthanized and the extent of protein oxidative damage, measured as protein carbonyls, was determined in homogenates of different brain regions.

Results: Age-related increases in carbonyl concentration were found in cortex, hippocampus, striatum and cerebellum of mice treated with the vehicle. The combination of TOC+CoQ, but not CoQ or TOC alone, decreased the carbonyl content in the old mice, most notably in the cortex but also in cerebellum and hippocampus.

Conclusions: This finding is in accordance with previous studies reporting improved learning in older mice following concurrent supplementation of these compounds (Free Radic Biol Med, 38, 729; 2005). The greater benefit associated with concurrent CoQ and TOC supplementation is consistent with the previous suggestion of a sparing-regenerative interaction between these compounds in the modulation of oxidative stress. (P01 AG022550, R01 AG027353)

Sponsor: *P01 AG022550, R01 AG027353*

101 *(Poster)*

Author: Parmeet Jodhka
Presenter: Parmeet Jodhka
Department: Pharmacology & Neuroscience
Classification: Postdoctoral Fellow/Resident

Parmeet Jodhka, David Lim, Wendy Underwood, and Meharvan Singh. Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107

PROGESTERONE INDUCES BDNF AND ERK 1/2 PHOSPHORYLATION VIA DISTINCT MECHANISMS IN EXPLANTS OF THE CEREBRAL CORTEX

Purpose: The higher prevalence of Alzheimer's disease in women relative to men has led to the suggestion that the precipitous decline in gonadal hormone levels following menopause may increase the risk for this disease. While considerable attention has focused on the consequence of estrogen loss, it is important to recognize that menopause results in a precipitous decline in progesterone (P4) levels as well. Thus, P4 may also be a relevant hormone to consider. Supporting the importance of this hormone, we have found that P4 is neuroprotective. Mechanistically, this protection required ERK activation, and was correlated with an induction of Brain-Derived Neurotrophic Factor (BDNF), a neurotrophin with known neuroprotective effects. Since P4 can elicit its effects via at least two receptor mechanisms, one involving an intracellular receptor (the classical PR) and the other involving the recently cloned membrane PR (mPR), the present study explored the involvement of these receptors in mediating the effects of P4 on ERK phosphorylation and BDNF expression in cerebral cortical explants which express both the classical PR and the mPR.

Methods: Organotypic explants from the cerebral cortex of postnatal day 3 mice were used to carry out the study. Treatments were applied on the 7th day in vitro. To assess the involvement of the classical PR or the mPR on progesterone induced ERK phosphorylation, explants were treated with P4, in the presence or absence of RU486, a classical PR antagonist; or the membrane-impermeable, BSA-conjugated progesterone, P4-BSA. Following different durations of treatment, explants were homogenized, and resulting lysates were assessed for the level of ERK 1/2 phosphorylation using Western blot analysis and a phosphospecific antibody to ERK 1/2. To determine whether the classical progesterone receptor or the mPR is involved in progesterone induced BDNF expression, explants were treated with RU486 or P4-BSA. Real-time quantitative RT-PCR was used to evaluate the relative level of BDNF mRNA in treated samples relative to the control samples. BDNF protein levels were assessed in explants using an enzyme-linked immunosorbent assay (ELISA).

Results: RU486 failed to inhibit the effect of P4 on ERK phosphorylation. However, RU486 successfully inhibited the ability of P4 to induce BDNF expression, supporting the likelihood that P4 induced increase in BDNF expression is mediated by the classical PR. To assess the involvement of the mPR, explants were treated with P4-BSA. P4-BSA mimicked the effects of P4 on ERK phosphorylation, suggesting that the membrane PR may be involved in this effect of progesterone. Interestingly, P4-BSA decreased expression of BDNF.

Conclusions: Collectively, our data support the involvement of two distinct receptor mechanisms in mediating the effects of P4 on ERK signaling and BDNF expression, two parameters relevant to the maintenance and promotion of cell viability.

Sponsor: *N/The National Institutes of Aging (NIA) - AG22550*

102 *(Poster)*

Author: Nopporn Thangthaeng
Presenter: Nopporn Thangthaeng
Department: Graduate School of Biomedical Sciences
Classification: GSBS Student
Nopporn Thangthaeng and Michael J. Forster University of North Texas Health Science Center, Fort Worth, TX 76107

SHORT-TERM GALACTOSE SUPPLEMENTATION EXACERBATES AGE-DEPENDENT MOTOR DEFICITS

Purpose: The purpose of this study was to determine if dietary (nonparenteral) supplementation of galactose would generate a similar accelerated aging model.

Methods: After 8-weeks, the mice were given behavioral tests to assess cognitive and motor performance. These tests included spontaneous locomotor activity, motor skills (wire suspension, elevated path test, rotorod test), the startle reflex and spatial swim maze learning.At the end of 14 weeks, brains of the mice were dissected into different regions for biochemical analyses to determine the extent of oxidative damage, as measured by protein carbonyls or TBARS, and the accumulation of AGEs, as indicated by receptor of glycosylation end product (RAGE) or carboxymethyllysine (CML) immunostaining.

Results: After 8-weeks, the mice were given behavioral tests to assess cognitive and motor performance. These tests included spontaneous locomotor activity, motor skills (wire suspension, elevated path test, rotorod test), the startle reflex and spatial swim maze learning. Galactose-supplemented groups had approximately a 4-fold increase in water intake and a significant reduction in weight when compared to the age-matched control. In old mice, galactose supplementation reduced locomotor activity and exacerbated age-related deficits in rotorod and auditory startle amplitude and reaction time. Interestingly, galactose-fed young mice showed increased auditory startle reactivity. There was no significant difference in spatial maze performance of the galactose-supplemented groups when compared to the control groups. Dietary galactose supplementation had no significant effect on oxidative damage or accumulation of AGEs in either of the age groups.

Conclusions: The results suggest that, in contrast to the effect of parenteral galactose, dietary galactose supplementation selectively targets neural systems involved in age associated motor dysfunctions. Therefore, the route of administration would appear to have a significant effect on the neurobiological consequences of galactose.

Sponsor: *Supported by NIH-NIA grant P01AG022550*

103 *(Poster)*

Author: James Hall
Presenter: Sonya Cornwell
Department: Psychology
Classification: GSBS Student
AUTHORS (FIRST NAME INITIAL LAST NAME): J. R. Hall1, S. Snyder1, 2, S. Cornwell1 INSTITUTIONS (ALL): 1. Medicine & Psychology, UNT Health Science Center, Fort Worth , TX, USA. 2. Lexicor Medical, Atlanta, GA, USA.

ENHANCING DEMENTIA DIAGNOSIS WITH EEG

Purpose: Purpose: The current study investigates the diagnostic predictive ability of neuropsychological assessment, magnetic resonance imaging, cardiovascular medical history and investigates the change in accuracy that occurs with the addition of electroencephalography (EEG).

Methods: Method: Participants were adults 50-85 who received healthcare from a geriatric outpatient clinic. The sample included normal adults and patients diagnosed with (AD), vascular dementia (VD), mixed dementia (AD/VD), and MCI. Dementia severity ranged from mild to severe. The clinical sample (n=111) comprised 78 normal adults and 33 patients cognitive impairment. Diagnosis was determined from previous medical records using history and physical, laboratory and imaging techniques, blood chemistry, MRI or CT and neurocognitive assessment using the ADAS-Cog. Cardiovascular risk factors were documented using medical history and questionnaire. EEG data were collected and digitized. Linear analysis of EEG was conducted using Fast Fourier Transform analysis. Non-linear analysis of EEG used the fractal dimension method with final estimate of the complexity being the average of the fractal dimensions of included epochs. Complexity at T5 was selected by stepwise logistic regression. Neuropsychological testing, medical history, and measurable biological data (EEG, MRI, CT) were analyzed to determine if they had sufficient independence from each other and added to the prediction of the geriatrician's diagnosis of cognitive functioning. The diagnostic categories were 'no diagnosis of cognitive impairment' (normal), (AD), (VD), mixed dementia (AD/VD), and (MCI). Sets of variables were analyzed using stepwise logistic regression.

Results: Results: Stepwise logistic regression revealed addition of cardiovascular risk factors to neuropsychological testing resulted in overall predictive accuracy of 80% (R2 = 0.53). Addition of non-linear analysis of EEG increased the overall accuracy to 92% (R2= 0.82). Neither linear EEG nor data from neuroimaging added to predictability

Conclusions: The results suggest that predictive accuracy in dementia diagnosis is enhanced with the use of relatively inexpensive neuropsychological tests, knowledge of cardiovascular history and non-linear analysis EEG data. The value of more expensive neuroimaging may be in the exclusion of specific structural causes when symptoms suggest their presence.

Sponsor: *Lexicor Medical*

104 *(Poster)*

Author: Ritu Shetty

Presenter: Ritu Shetty

Department: Pharmacology & Neuroscience

Classification: GSBS Student

R.A. Shetty, N. Sumien, and M.J. Forster. Dept of Pharmacology and Neuroscience, UNTHSC, Fort Worth, Texas- 76107

DIETARY SUPPLEMENTATION OF ?-TOCOPHERYL ACETATE AND COENZYME Q10 IN AGED MICE IMPROVES SELECTED ASPECTS OF COGNITIVE AND PSYCHOMOTOR FUNCTION

Purpose: The goal of the proposed study was to determine if the antioxidants, coenzyme Q10 (CoQ) and a-tocopheryl acetate (Toc), act in synergy to ameliorate age-related impairments in cognitive and psychomotor performance.

Methods: Separate groups of male C57BL/6 young mice aged 3-4 or 17-18 months (total n=124) were fed, for a period of 15 weeks, either a control diet, or diets supplemented with (i) (+) a-tocopheryl acetate (200 mg/kg body wt/day), (ii) CoQ (148 mg/kg body wt/day), or (iii) the combination of these supplements. Beginning four weeks after implementation of the diets, the mice were tested for their ability to perform on an age-sensitive battery of tests for cognitive and psychomotor function.

Results: The results indicated that there was a significant improvement in performance of the old mice in a test of coordinated running (rotorod) and of learning ability (active avoidance), following supplementation with Toc alone, and following the combination of CoQ and Toc. The improvements in active avoidance learning were most robust when the CoQ and Toc were supplemented in combination. Little or no improvement was observed following any treatment in a test of spatial learning dependent on hippocampal function, and no effects were observed in a number of other tests of psychomotor and sensory function.

Conclusions: Overall, these results suggest that short-term supplementation of antioxidants may be effective in improving selected aspects of age-related cognitive and psychomotor decline. The improvements in active avoidance learning may be attributable to a synergistic effect of concurrent Toc and CoQ supplementation, whereas improvement of psychomotor function may be attributable to supplementation of Toc alone.

Sponsor: *P01 AG022550, RO1 AG027353*

105 *(Oral)*

Author: Courtney Bowles

Presenter: Courtney Bowles

Department: Pharmacology & Neuroscience

Classification: GSBS Student

Courtney A. Bowles, Wendy Underwood, Parmeet Jodhka, and Meharvan Singh University of North Texas Health Science Center at Fort Worth, Department of Pharmacology and Neuroscience, Fort Worth, TX 76107

PROGESTERONE-INDUCED REGULATION OF NEUROTROPHINS IN C6 CELLS

Purpose: The purpose of this work is to further characterize the role of the mPR in progesterone's neuroprotective abilities and to identify signaling pathways critical for its effect.

Methods: Experiments were carried out in two different model systems. Cerebral cortical explants (brain slices) from postnatal day three mice were used in addition to the rat C6 glioma cell line. Cells were treated with 100nM progesterone (P4) or the membrane impermeable, bovine serum albumin (BSA) conjugated steroid, P4-BSA. Immunoblotting techniques were used to identify activated/phosphorylated signaling proteins. The Enzyme-Linked Immuno-Sorbent Assay (ELISA) was employed to quantify both intracellular levels of neurotrophin and the levels in the media which correspond to the neurotrophin that has been released from the cells. Lactate dehydrogenase (LDH) assays were performed as a measure of cell death in response to a glutamate insult in the presence and absence of progesterone and 6nM K252a, a pan- inhibitor of Trk receptors.

Results: Our data show that activation of the mPR elicits activation of the ERK/MAPK pathway. Activation of this signaling pathway is not inhibited with the use of the classical PR antagonist RU486. Inhibition of the ERK/MAPK pathway using U0126 in the presence of P4 causes an increase in the amount of intracellular nerve growth factor (NGF) relative to both control and P4 treated C6 cells. Inhibition of Trk receptors also prevented the protective effect of P4 against glutamate induced excitotoxicity.

Conclusions: We conclude that inhibition of the ERK/MAPK pathway (via the mPR) prevented the release of synthesized neurotrophin (an effect mediated by the classical PR). As such, the net effect was an increase in cellular accumulation of the neurotrophin. These data define the importance of the ERK/MAPK pathway in the regulation of neurotrophin release, and may offer unique insight into the development of novel therapeutic strategies for the treatment of menopausal symptoms and age-related cognitive disorders such as Alzheimer's disease.

Sponsor: *NIA: AG22550*

106 *(Poster)*

Author: Craig Hilburn

Presenter: Craig Hilburn

Department: Pharmacology & Neuroscience

Classification: GSBS Student

Craig R. Hilburn, Michael J. Forster, and George King Department of Pharmacology and Neuroscience, University of North Texas, Health Science Center Fort Worth, Tx. 76107

LONG TERM EFFECTS OF CHRONIC PSYCHOSTIMULANT ADMINISTRATION

Purpose: Previous research has demonstrated that chronic psychostimulant abuse results in long lasting alterations in numerous brain systems. In particular, such alterations involve major dopaminergic pathways in the brain, which are also selectively vulnerable to oxidative stress and aging. Thus, the current studies addressed the hypothesis that exposure to psychostimulants could interact with brain aging processes, leading to accelerated loss of behavioral functions dependent on dopaminergic systems.

Methods: A mouse model of chronic psychostimulant abuse was used in which 10- month-old mice were exposed to a 28-day, continuous infusion of cocaine (40 mg/kg/day), methamphetamine (2 mg/kg/day), or saline. After discontinuation of the treatment, separate groups of the mice were tested at 11, 14, or 16 months of age i.e., 14, 90, or 150 days following the treatment.

Results: Both cocaine and methamphetamine produced a deficit in psychomotor function at 11 months of age. This deficit was evident in a wire-suspension test which requires muscle strength, and in elevated bridge and rotorod tests requiring balance and sensorimotor coordination. The wire-hanging deficit of the cocaine-exposed mice recovered to the performance level of the age-matched controls at 16 months of age, while methamphetamine treated mice continued to show impairments. At 16 months of age neither psychostimulant treatment group showed any recovery of function on the elevated bridge test or rotating rod tests. Neither cocaine nor methamphetamine exposure was associated with cognititive deficits as tested in a Morris water maze.

Conclusions: Taken together the current studies provide evidence that psychostimulant exposure in late adulthood may produce persistent impairments in psychomotor function. While these impairments are significant, the current studies did not provide clear evidence that psychostimulant exposure produced an acceleration of normal brain aging.

Sponsor: *R36 AG029004-01, P01 AG022550*

107 *(Poster)*

Author: Sarah Ross

Presenter: Sarah Ross

Department: Family and Community Medicine

Classification: Dual Degree Student DO/MS

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THE INFLUENCE OF RESIDENCE AND FAMILY ON THE RATE OF DECLINE IN PATIENTS WITH DEMENTIA

Purpose: The purpose of this study is to determine the relationship between a patient's marital status, number of relatives, and place of residence on the rate of decline of their dementia as measured by serial Mini-Mental Status Exam scores. Data on patients' marital status, and number of family members may help determine how much social support and interactions the patient enjoys. The patent's residence helps indicate the level of care that patient needs, whether they are independent at home or requiring full assistance in a nursing home. These family and home situations may effect the patient's disease progression. Understanding social factors that influence the progression of dementia will help physicians better treat and counsel patients and families.

Methods: This is a database study using the Clinical Database for Dementia, available through the Department of Geriatrics at the University of North Texas Health Sciences Center. All patients in the database with a clinical diagnosis of dementia were included. The variables marital status, number of relatives, and residence were compared with mini-mental status exam scores.

Sponsor: *N/A*

108 *(Poster)*

Author: Kimberly Brown

Presenter: Kimberly Brown

Department: Pharmacology & Neuroscience

Classification: Staff

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ROLE OF ETHNICITY IN THE FEATURES OF HOT FLASHES IN MENOPAUSAL WOMEN

Purpose: A determination of the ethnic basis of the features of hot flash experience during menopause has not been reported and will contribute to our understanding of health disparities in middle-aged women. The goal of this clinical study was to determine whether there were differences in the number, length and bothersomness of hot flashes between Caucasian (n=82) and non-Caucasian (n=29) women

Methods: Women aged 40 to 60 were invited to participate. A total of 111 women participated in this study. During the study session, the Menopausal Vasomotor Symptom (MVS) survey was administered to all study participants. Data were collected on MVS survey, demographic variables, and BMI.

Results: The majority (85%) of Caucasian women had current or past hot flashes. Most had less than 5 hot flashes per day (72%), and they lasted less than 5 minutes (66%). More intense hot flashes were perceived by 38% of Caucasian women, and 60% had interruption of sleep. For non-Caucasian women, 86% experienced hot flashes currently or in the past. Forty two percent of non-Caucasian women experienced more than 5 hot flashes per day, and they lasted less than 5 minutes (in 58% of women). More intense hot flashes were perceived by 58% of non-Caucasian women, and 89% had interruption of sleep.

Conclusions: Even in this small sample, we observed a significant difference between non-Caucasian and Caucasian women for the number of hot flashes per day and disturbance in sleep ($p<0.005$). These findings suggest a trend for more frequent, more intense, and more bothersome hot flashes in non-Caucasian women in comparison to Caucasian women.

Sponsor: *N/A*

109 *(Poster)*

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Presenter: Sandra Longoria

Department: Pharmacology & Neuroscience

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ANGELA PETERSON-FORD UNTHSC PETER KOULEN UNTHSC

THE EFFECTS OF OXIDATIVE STRESS ON IP3 RECEPTOR FUNCTION IN THE NEURONAL CELL LINE HT22

Purpose: Purpose: Oxidative stress contributes to the onset of neurodegenerative disorders such as Alzheimer 's disease (AD) and stroke. Oxidants such as, tert-butyl hydrogen peroxide (tBHP), have been used in vitro to induce oxidative stress in neurons. Previous research has shown that subtle changes in the regulation of intracellular calcium (Ca2+) contribute to brain aging and increase vulnerability of neurons to neurodegenerative disease processes. Inositol 1, 4, 5-trisphosphate (IP3), is a second messenger generated at the plasma membrane. IP3 receptors (IP3R) are located on the endoplasmic reticulum (ER) membranes that diffuse into the cytosol and play an important role as intracellular calcium channels (ICC) releasing Ca2+ in response to their ligand IP3. The purpose of the present study is that oxidative stress induced with tBHP causes increased intracellular Ca2+ release via activation of IP3 receptors.. Hypothesis: We tested the hypothesis that ICCs contribute critically to Ca2+ dysregulation in neurons experiencing oxidative stress.

Methods: Method: We used the murine hippocampal cell line HT22, as a model for neuronal oxidative stress. HT22 cells express IP3 and its receptor (IP3R). We induced oxidative stress with an overnight incubation of tBHP in cultured HT22 cells and we performed immunocytochemistry and Ca2+ imaging experiments to show the areas of IP3R expression and activity under normal conditions and induced oxidative stress.

Results: Results: tBHP treatment increased IP3 receptor expression and Ca2+ release activity.

Conclusions: Conclusion: Our findings support that treatment with tBHP negatively affects regulation of Ca2+ release through increased expression and activity of IP3 receptors.

Sponsor: *N/A*

200 *(Poster)*

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Presenter: Rusha Thomas

Department: Molecular Biology and Immunology

Classification: GSBS Student

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HIF: A KEY SURVIVAL FACTOR FOR PROSTATE CANCER CELLS DURING STRESS OF PROLONGED SERUM DEPRIVATION

Purpose: The objective of this study is to assess the role of HIF-1a in promoting prostate cancer cell survival during stress of prolonged serum deprivation, and will elucidate the signal transduction pathway involved in sensing growth factor depletion and consequently upregulating HIF-1a protein synthesis in prostate cancer cells.

Methods: Western blot analysis was employed to determine protein levels of HIF-1a, phospho-Akt and total-Akt. Cell viability was assessed by trypan blue assay. HIF-1a mRNA levels were assessed by RT-PCR. Depletion of HIF-1a was achieved by transfecting the cells with siRNA against HIF-1a. PI3K activity was inhibited by treatment with the pharmacological inhibitor of PI3K, LY294002. PTEN was overexpressed by transfection of the PC-3 cells with a PTEN expression vector.

Results: Our studies in PC-3 prostate cancer cells reveal that HIF-1a protein levels increase as the duration of serum deprivation increases. However, there is no significant change in HIF-1a mRNA levels during serum deprivation. In addition, the increase in HIF-1a protein levels during serum deprivation correlated with increased cell survival. Silencing of HIF-1a expression with siRNA resulted in a decrease in cell number during serum deprivation. Pharmacological inhibition of PI3K with LY294002 dramatically decreased cell number, and HIF-1a protein levels during serum deprivation. In addition, transfection of the PTEN-deficient PC-3 cells with PTEN expression vector markedly decreased HIF-1a protein expression and cell number during serum deprivation. This result is consistent with our previous result, because PTEN is a negative regulator of PI3K activity. Prolonged serum deprivation also dramatically increased the levels of phospho-Akt, Akt being the downstream target of PI3K.

Conclusions: Our studies demonstrate for the first time that prolonged serum deprivation upregulates HIF-1a via the PI3K pathway, and this increase in HIF-1a protein levels markedly increases cell survival during this stress situation.

Sponsor: *N/A*

201 *(Poster)*

Author: Art Braden

Presenter: Art Braden

Department: Molecular Biology and Immunology

Classification: Postdoctoral Fellow/Resident

Arthur R. Braden Ph.D. Department of Molecular Biology and Immunology The University of North Texas Health Science Center

POLYMERIC NANOPARTICLES FOR IN VITRO AND IN VIVO SUSTAINED REDUCTION OF ANNEXIN A2 AS ADJUVANT PROSTATE CANCER THERAPY

Purpose: High levels of annexin A2 expression have been positively associated with cancers of lung, pancreas, breast, brain and prostate tumors. Prostate cancer is characterized by an absence of detectable annexin A2 expression during pre-neoplastic stages; however in advanced malignant stages annexin A2 expression is regained. Thus, loss of annexin A2 modulation in prostate cancers indicates a potentially important regulatory role for this protein in prostate cancer progression.

Methods: We previously reported on the use of a nanotechnology-based, non-viral controlled-release delivery system utilizing pDrive-sh AnxA2 plasmid DNA to regulate the function of annexin A2 in vitro and in vivo. These nanoparticles are formulated from an FDA-approved, biodegradable and biocompatible polymer, poly (DL-lactide-co-glycolide) (PLGA), which undergoes slow intracellular hydrolysis to release the therapeutic agent at a sustained rate.

Results: We observe a reduction in both cellular proliferation and migration in vitro upon exposure of metastatic prostate cancer cells to pDrive-sh AnxA2 loaded nanoparticles. As an extension of this work the in vivo efficacy of our nanoparticle system was investigated using BALB/C athymic nude mice. Xenograft prostate tumors were induced using the metastatic prostate cancer cell line DU-145. We find that upon administration of pDrive-sh AnxA2 loaded nanoparticles there is a reduction in tumor volume, and protein levels of both annexin A2 and VEGF. Our results also demonstrate a significant increase in the survival times of pDrive-sh AnxA2 nanoparticle treated mice with xenograft tumors over control or blank unloaded nanoparticles

Conclusions: The use of pDrive-sh AnxA2 loaded nanoparticles may be clinically relevant as an adjuvant therapy to current clinical treatment regimens for prostate cancers. (Research supported by a grant R21CA109593 from the National Institutes of Health)

Sponsor: *N/A*

202 *(Poster)*

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POLY LACTIC-CO-GLYCOLIC ACID NANOPARTICLES FOR THE TREATMENT OF ACTUE PROMYELOCYTIC LEUKEMIA

Purpose: Acute Promyelocytic Leukemia (APL),a subtype of Acute Myeloid Leukemia, is characterized by t(15;17) reciprocal chromosomal translocation resulting in the formation of a chimeric promyelocytic leukemia/retinoic acid receptor-alpha(PML/RARa) fusion protein. PML/RARa fusion protein blocks myeloid differentiation and suppresses apoptosis resulting in clinical expression of cells in a promyelocytic stage. A primary treatment option for patients suffering from APL is the use of the drug all-trans-retinoic acid (ATRA), a derivative of vitamin A. ATRA induces differentiation in various cancers including APL. The chemical properties of ATRA lead to limited treatment options and frequently lead to drug resistance and disease relapse.

Methods: Using a nano-dispersion technique we encapsulated ATRA in polymeric nanoparticles for sustained intracellular release of the drug and enhanced cellular uptake.

Results: The formulation technology successfully encapsulates 90% of the drug, generating nanoparticles in a size range from 61-578nm. ATRA release from nanoparticles follows classical small molecule dynamics. Our preliminary results show rapid uptake of the particles by NB4 cells, an APL cell line. Nanoparticles loaded with 0.3 μM of ATRA induced differentiation of NB4 cells at similar rates as the standard 1.0 μM dose of free drug.

Conclusions: Our preliminary data indicate that the properties of these nanoparticles may provide a more efficacious treatment for APL than the existing standard of care.

Sponsor: *N/A*

203 *(Poster)*

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Presenter: Cherice Roth
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Classification: GSBS Student
Cherice P Roth - UNTHSC Jamboor K Vishwantha - UNTHSC

HORMONE REFRACTORY PROSTATE CANCER: ALTERNATE PROSTATE SPECIFIC ANTIGEN PATHWAYS

Purpose: The American Cancer Society estimates that at least 230,110 men in the United States will be diagnosed with prostate cancer this year and that 29,900 men died of the disease in 2004. However the ethnicity of diagnosed patients is not evenly distributed. African American males are 50-60% more like to be diagnosed with prostate cancer. This is higher than any other ethnic background according to the Center for Disease Control and prevention (CDC) 55% of African American men that have been diagnosed with invasive prostate cancer will die from the disease. It has been shown that African Americans have several single nucleotide polymorphisms (SNPs) in interleukin 4 (IL4) that have been associated with the modulation of IgE gene transcription via the STAT6 pathway. IL4 has been found is high levels in and around the microenvironment of tumors and in fact aids in immunosurveillance and eventual clearance of some tumors. IL4 has also been linked to the transcription of prostate-specific antigen (PSA) via androgen receptor and the PI3 kinase pathway. It is hypothesized that there is an alternative pathway for the transcriptional activation and subsequent secretion of PSA. The focus of this study is to identify the alternate PSA secretion pathway.

Methods: The methods used to determine this alternate pathway are RT-PCR, ELISA, and SiRNA of STAT6.

Results: Upon cell line stimulation with IL-4 and LY294002 (a PI3Kinase inhibitor) two very different stories emerge. RT-PCR and PSA ELISAs reveal that when IL-4 in the present is presented to low passage androgen dependent LnCap cells PSA levels decrease in a dose dependent manner. However this decrease is not seen in PC-3 and LnCap C42 cells this is significant because the latter cell lines are androgen dependent. There is also a significant increase in PSA mRNA when STAT6 is downregulated via SiRNA.

Conclusions: These data indicate that there is an alternate pathway for the genetic control of PSA.

Sponsor: *NIH*

204 *(Oral)*

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INVOLVEMENT OF P70S6K IN CISPLATIN-MEDIATED CELL DEATH

Purpose: p70S6K, a serine/threonine kinase regulates the progression of cells from the G0 to G1 phase of cell cycle by translational upregulation of a family of mRNA transcripts. The activation of p70S6K and its downstream target ribosomal protein S6 mediates nutrient and mitogen stimulated translation. It has been shown that p70S6K is constitutively phosphorylated and activated in lung cancer cells. Rapamycin which blocks p70S6K activation is already in clinical trials for chemotherapy, suggesting that p70S6K is an attractive target for therapy. Cisplatin is a DNA damaging agent widely used for the treatment of many cancers, including lung cancers. Many chemotherapeutic drugs, including cisplatin kill cancer cells by inducing apoptosis. Activation of caspases, a family of cysteine proteases, is central to apoptotic cell death. The purpose of this study is to determine the role of p70S6K in cisplatin-induced apoptsis in human lung cancer cells.

Methods: Human lung cancer H69 and A549 cells were treated with cisplatin for varying time period. p70S6K protein and phosphorylation levels were detected by immunoblotting using total and phospho-specific antibodies. Apoptosis was monitored by the cleavage of poly-ADP-ribose-polymerase (PARP). Caspases and p70S6K were depleted by transfecting cells with siRNA.

Results: Cisplatin treatment caused a time- and concentration-dependent decrease in the levels of p70S6K in H69 and A549 cells. Depletion of p70S6K by siRNA resulted in a decrease in cisplatin-induced PARP cleavage. Pretreatment of cells with the broad specificity caspase inhibitor ZVAD prior to cisplatin treatment reversed cisplatin induced cleavage of PARP as well as of p70S6K. Cell-permeable peptide inhibitors of capsase-3 but not caspase-2, -8 or -9 inhibited cisplatin-induced protelytic cleavage of p70S6K. Furthermore, depletion of caspase-3 by siRNA blocked the cleavage of p70S6K. Depletion of caspase-2, -8, -9 did not inhibit the down-regulation of p70S6K by cisplatin. Finally, cisplatin failed to induce cleavage of p70S6K in MCF-7 cells that lack functional caspase-3.

Conclusions: We show for the first time that p70S6K is a substrate for caspase-3. Our results suggest that proteolytic cleavage of p70S6K may be important for DNA damage-induced apoptosis.

Sponsor: *N/A*

205 *(Poster)*

Author: Eswar Shankar
Presenter: Eswar Shankar
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Classification: Postdoctoral Fellow/Resident
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DOWNREGULATION OF P53 BY PKC EPSILON OVEREXPRESSION IS ASSOCIATED WITH TRAIL RESISTANCE.

Purpose: Protein kinase C (PKC) is a family of phospholipid-dependent serine/threonine kinases that play an important role in cell proliferation and cell death. We have recently reported that PKCe is an anti-apoptotic protein that inhibits TRAIL-induced apoptosis in MCF-7 breast cancer cells. The p53 pathway has been shown to be an important regulator of Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) induced apoptosis. Mdm2 is a negative regulator of p53. Phosphorylation of mdm2 at ser166 and ser188 by Akt results in its activation. Active mdm2 translocates to the nucleus and binds to p53 thereby inhibiting its transcriptional activity. The mdm2-p53 complex relocates to the cytoplasm where mdm2 acts as an E3 ubiquitin ligase causing the proteasomal degradation of p53. In the present study, we examined the involvement of p53 and mdm2 in PKCe-mediated TRAIL resistance.

Methods: MCF-7 human breast cancer cells overexpressing PKC? (MCF-7/PKCe) or empty vector (MCF-7/Neo) were treated with or without TRAIL. Western blotting was used to compare levels of p53, mdm2, phospho-mdm2 (Ser166) and cleavage of PARP (a measure of apoptosis). mRNA levels of p53 and mdm2 were determined by RT-PCR. Akt, p53, mdm-2 and PKCe were depleted by transfecting cells with specific siRNA.

Results: Ectopic expression of PKCe in MCF-7 cells was associated with a decrease in p53 and a concomitant increase in mdm-2 at both the protein and mRNA level. Levels of phospho-mdm2 (Ser166) were also higher in the MCF-7/PKCe cells compared to MCF-7/Neo cells. Silencing p53 using siRNA abrogated TRAIL-induced cell death in both MCF-7/Neo and MCF-7/PKCe cells whereas depletion of mdm-2 sensitized MCF-7/PKCe cells to TRAIL. It has been shown that Akt regulates p53. Furthermore, we have previously shown that Akt acts downstream of PKCe and mediates survival of MCF-7/PKCe cells. Therefore, we determined the effect of Akt depletion on p53 levels. Knockdown of Akt in MCF-7/Neo and MCF-7/PKCe cells was associated with an increase in p53, a decrease in phospho-mdm2 and enhanced cell death.

Conclusions: These results suggest that PKCe mediates TRAIL resistance by Akt-mediated activation of mdm-2 causing downregulation of p53. (This work was supported by the grant CA71727 from the NCI.)

Sponsor: *NCI*

206 *(Poster)*

Author: Kelly Ylitalo

Presenter: Kelly Ylitalo

Department: Epidemiology

Classification: SPH Student

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PREMENOPAUSAL BREAST CANCER MORTALITY IN THE UNITED STATES, 1999-2003: TEMPORAL TRENDS OF RACIAL AND URBANIZATION STATUS DISPARITIES

Purpose: Objective: Few studies have addressed variation of premenopausal breast cancer mortality between metropolitan and non-metropolitan areas. We characterized recent temporal trends of racial and urbanization status disparities for premenopausal breast cancer mortality in the United States (US).

Methods: Methods: We queried CDC WONDER to obtain breast cancer mortality data for women ages 20-44 years for 1999 and 2003 from the 1999-2003 Compressed Mortality File (CMF). The CMF classified underlying cause of death according to International Classification of Diseases 10 criteria (breast cancer: C50) from all 50 states and Washington, DC. Data were stratified by race (Non-Hispanic Black or Non-Hispanic White) and urbanization status (large central metropolitan or non-metropolitan [micropolitan]). Mortality rate ratios (MRRs) were used for comparisons.

Results: Results: The premenopausal breast cancer MRR between Black and White females in the US declined from 1999-2003 (1999 MRR=2.42, 2003 MRR=2.07). Stratified data indicated that the MRR between Black and White females declined for US metropolitan areas (1999 MRR=2.52, 2003 MRR=2.15) and US non-metropolitan areas (1999 MRR=2.05, 2003 MRR=1.73). The MRRs between metropolitan and non-metropolitan areas (Black and White combined) increased slightly from 1999-2003 (1999 MRR=1.08, 2003 MRR=1.18).

Conclusions: Conclusion: Our data suggest that racial disparities for premenopausal breast cancer mortality in the US have collectively improved from 1999-2003, but urbanization status disparities increased. The results should be interpreted cautiously because of the brief period of investigation. More importantly, our data suggest that the interaction of race and urbanization status may be a consideration for future epidemiologic studies of premenopausal breast cancer.

Sponsor: *N/A*

207 *(Poster)*

Author: Usha Sivaprasad

Presenter: Usha Sivaprasad

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Classification: Postdoctoral Fellow/Resident

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EATING ONESELF TO DEATH.....OR LIFE? THE STORY OF A MEK INHIBITOR

Purpose: Autophagy (self-eating) is a process of degradation of cytoplasmic components in lysosomal compartments called autophagosomes. Autophagy has received recent attention as a novel programmed cell death pathway. Autophagy can prolong cell survival in stressful conditions and is therefore implicated in resistance to chemotherapy, while unchecked autophagy results in cell death. Tumor necrosis factor-alpha (TNF), used as chemotherapeutic agent, induces both apoptosis and autophagy in cancer cells. Intracellular signaling mechanisms that regulate TNF-induced autophagy are unclear. The p70S6 kinase (S6K) pathway has been linked to starvation-induced autophagy. The mitogen-activated protein kinase (MAPK) pathway regulates cell survival and is also implicated in autophagy. Furthermore, there may be cross-talk between these pathways. Therefore, the aim of the present study was to delineate the role of S6K and MAPK in TNF-induced autophagy in human breast cancer MCF-7 cells.

Methods: Western blotting was used to determine PARP cleavage (a measure of apoptosis) and conversion of the autophagy marker protein LC3-I to LC3-II. To examine the association of LC3-II to autophagosomes, MCF-7 cells were stably transfected with a vector expressing EGFP alone or EGFP-tagged human LC3. U0126 and rapamycin were used to inhibit MAPK kinase (MEK1/2) and S6K activity, respectively. MEK1, MEK2 and S6K were depleted by transfecting MCF-7 cells with siRNA.

Results: TNF stimulated a time-dependent phosphorylation of S6K and MAPK in MCF-7 cells. TNF induced autophagy as evidenced by an increase in LC3-II levels and in the number of cells displaying LC3-GFP associated with autophagosomes. The MEK1/2 inhibitor U0126 attenuated autophagy but enhanced apoptosis induced by TNF. Depletion of either MEK1 or 2 by siRNA augmented TNF-induced PARP cleavage. Depletion of MEK1, but not MEK2, increased LC3-II levels. Inhibition of S6K activity using rapamycin or depleting S6K by siRNA decreased TNF-induced LC3-II levels, but had little effect on PARP cleavage. Interestingly, U0126 also attenuated basal and TNF-induced S6K phosphorylation. MEK1 or -2 depletion, however, did not affect S6K phosphorylation.

Conclusions: Taken together, these data suggest that while U0126 enhanced apoptosis by inhibiting MEK1/2, it suppressed autophagy by inhibiting S6K.

Sponsor: *This work was supported by an NCI grant CA71727 to A.B.*

208 *(Poster)*

Author: Maya Nair

Presenter: Maya Nair

Department: Molecular Biology and Immunology

Classification: Staff

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A LIPOPROTEIN BASED NOVEL DRUG DELIVERY SYSTEM AS A TOOL TO OVERCOME STRESS INDUCED IMMUNE SUPPRESSION DURING CANCER CHEMOTHERAPY

Purpose: The long-term goal of this project is to develop innovative and comprehensive approaches to treat cancer. Radiation and chemotherapy are helpful to treat cancer, but produce deleterious side effects, including immunosupression. The immunosuppressive action of chemotherapeutic agents can lead to secondary infections and drug resistance creating further complications. RLIP76, a non-ABC transporter is a key protein in stress defense pathway that also facilitates drug resistance in cancer cells. RLIP76 and HDL receptors are over expressed in resistant neoplasms, targeting these cancers with rHDL/drug complex is a novel and potentially powerful approach for enhanced drug delivery to cancer cells while preserving the normal cells' immune response.

Methods: Preliminary studies were carried out by preparing rHDL/drug complexes that were characterized with regard to their lipid/protein and drug contents and their cytotoxicity toward ovarian cancer cells. The drug selected for the study is AD32 (Valrubicin), a chemotherapeutic agent, structurally similar to adriamycin (doxorubicin). Even though AD32 has been found to be much less toxic to normal tissues than doxorubicin, its therapeutic application has been restricted to non-systemic routes due to its poor water solubility. Cellular uptake studies were done to evaluate the involvement of RLIP in the uptake of rHDL/valrubicin complex. In vitro and in vivo studies were done to evaluate the effect of rHDL drug delivery system on the immune cell population.

Results: rHDL particles containing valrubicin had a consistent composition, molecular weight and exceptional stability upon ultracentrifugation and gel chromatography. Data on the uptake of valrubicine suggesting that the uptake were facilitated by receptor-mediated mechanism. Additional studies on the cell viability of the immune cell population showed that valrubicin delivered as a component of rHDL is much less cytotoxic to the immune population at the same time the cytotoxicity with the ovarian cancer cell showed enhanced cytotoxicity for rHDL/ ValR.

Conclusions: 1) rHDL/valrubicin particles have been prepared with substantially higher valrubicin content.2) The rHDL/ValR formulation showed markedly higher cytotoxicity against ovarian cancer cells and showed much less cytotoxicity towards the immune cells compared to the free drug.

Sponsor: *N/A*

209 *(Poster)*

Author: Roberto Cardarelli

Presenter: Roberto Cardarelli

Department: Research

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UNFAIR TREATMENT DUE TO RACE NOT ASSOCIATED WITH COLORECTAL CANCER TEST USE

Purpose: Individuals belonging to racial and ethnic minority groups have higher rates of colorectal cancer (CRC) mortality and lower rates of screening. Although various psychosocial factors have been studied as predictors for CRC screening, no study has assessed the impact of unfair treatment due to race on adequate CRC test use.

Methods: Data from the 2004 Behavioral Risk Factor Surveillance System (N=17,604) were used to assess the impact of differential treatment due to one's race on adequate CRC test use. Logistic regression was used to assess the relationship between adequate CRC testing and unfair treatment due to one's race.

Results: Unfair treatment due to race was not associated with adequate CRC test use after controlling for potential confounders (African Americans: OR, 0.86; 95% CI, 0.71-1.07; Hispanics/ Latinos: OR, 1.44; 95% CI, 0.82-2.55). Age, having a personal healthcare provider, and education were significant predictors for all racial/ethnic groups. Among whites, health insurance status and income were associated with adequate CRC test use. Among African Americans and Hispanics/Latinos, having a personal healthcare provider was a significant predictor (African Americans: OR, 6.11; 95% CI, 2.83-13.19; Hispanics/ Latinos: OR, 4.77; 95% CI, 1.08-18.44).

Conclusions: Unfair treatment due to race is not associated with adequate CRC test use, whereas having a personal healthcare provider and education were significant factors for all ethnic/racial groups.

Sponsor: *N/A*

210 *(Poster)*

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OVEREXPRESSION OF PKC-ETA CONFERS TRAIL RESISTANCE IN BREAST CANCER CELLS

Purpose: Protein kinase C (PKC)-eta, a member of the PKC family, plays a major role in cell proliferation and differentiation, especially in epithelial cells. PKC-eta protein levels are upregulated in breast tumors. Therefore, targeting PKC-eta downregulation is an important strategy in breast cancer therapy. Tumor-necrosis factor alpha-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family which is selective in killing cancer cells while sparing normal cells. The overall objective of this project is to test the hypothesis that depletion of PKC-eta sensitizes breast cancer cells to TRAIL-induced cell death.

Methods: MCF7 cells were stably transfected with empty vector (pcDNA3) or vector containing PKC-eta construct to determine the effect of PKC-eta overexpression on TRAIL-induced cell death. Protein levels were determined by Western blot analysis. Cell death was monitored by the cleavage of poly(ADP-ribose)polymerase(PARP). Cell viability was determined by the colorimetric MTT assay. We inhibited PKC-eta using a general PKC inhibitor bisindolylmalemide (BIM) and employed siRNA technology to test the effect of PKC-eta and PKC-epsilon depletion on TRAIL-mediated cell death.

Results: 1806 cells derived from patients at early stage breast cancer expressed low levels of PKC-eta and were sensitive to TRAIL-induced cell death. In contrast, 1428 cells derived from patients at late stage breast cancer contained high levels of PKC-eta and were highly resistant to TRAIL. MCF7 cells, an established breast cancer cell line, expressed intermediate levels of PKC-eta and were moderately sensitive to TRAIL-mediated cell death. Ectopic expression of PKC-eta in MCF7 cells resulted in resistance to TRAIL-induced cell death. Depletion of PKC-eta alone by siRNA was sufficient to cause cell death in MCF7 cells. Although PKC-specific inhibitor BIM downregulated PKC-eta and sensitized 1428 cells to TRAIL-induced cell death, depletion of PKC-eta by siRNA did not reverse TRAIL resistance. 1428 cells contained high levels of another anti-apoptotic PKC, namely PKC-epsilon. Knockdown of PKC-epsilon sensitized cells to TRAIL-induced cell death.

Conclusions: Overexpression of PKC-eta in breast cancer cells was associated with resistance to TRAIL-induced apoptosis. However, depletion of PKC-eta alone in late stage breast cancer cells was not sufficient to reverse TRAIL resistance, presumably because the presence of other anti-apoptotic proteins, such as PKC-epsilon could contribute to TRAIL resistance.

Sponsor: *NIH/NCI CA71727 and NSF Project SCORE grants*

211 *(Poster)*

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MOLECULAR UNDERPINNINGS OF BREAST CARCINOMA IN THREE DIMENSIONAL CULTURES

Purpose: The microscopic features of epithelial cells in mammary glands such as polarity, lumen formations etc. are crucial for their normal functioning. This well ordered arrangement is altered in most breast tumors. However, very little is known about the genetic machinery that induces these changes. The myriad of experiments conducted on monolayer cultures, to fathom the epithelial tumor pathogenesis, are not completely bonafide, as monolayer cultures do not recapitulate the glandular microenvironment. The cultures in 3D have, however, been shown to mimic the acinar phenotype. Moreover, increasing evidences suggest that oncogenes exhibit different behaviours in 3D cultures and in monolayers. Hence, we specifically aim to grow MCF10A cells, a normal counterpart of MCF-7 breast cancer cells, in 3D culture and establish stable pools of cells expressing genes of interest. Then we would try to validate previous findings from our laboratory that protein kinase C (PKC) e functions as an anti-apoptotic protein in breast cancer cells. Eventually, we intend to delineate the roles of apoptosis and autophagy in tumor formation.

Methods: Cells for 3D would be cultured on reconstituted basement membranes by overlay method. Stable pools of MCF-10A cells expressing genes of interest, would be established by retroviral mediated gene transfer. Confocal microscopy of 3D cultures and imuunofluorescent techniques would be used for morphological analysis. Western blotting and FACS would be used for assessessment of protein expression levels and for cell sorting respectively.

Results: Our lab has previously shown that PKCe activates protein kinase B (PKB)/Akt via DNA-dependent protein kinase (DNA-PK) to protect breast cancer cells against tumor necrosis factor-alpha (TNF) induced cell death. We anticipate that introduction of constitutively active PKC-e either alone or in combination with Akt in MCF-10A will disrupt architecture of normal epithelial MCF-10A cells.

Conclusions: Deviation from well-arranged architecture is hallmark of mammary gland tumors. 3D culture represents the optimal system for unraveling the genetic events involved in mammary tumor pathogenesis. Our work is expected to augment the studies on signaling cascade in a physiologically more relevant system.

212 *(Poster)*

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CISPLATIN-INDUCED APOPTOSIS IS MEDIATED BY CASPASE-2

Purpose: Cis-diamminedichloroplatinum (II) (cisplatin) is one of the most important anticancer drugs used in the treatment of solid tumors. Cisplatin causes DNA damage by interacting with DNA and leads to apoptosis. Caspases, a family of cysteine proteases that cleave proteins after aspartic acid residues, are essential for the induction of apoptosis. Initiator caspases activate effector caspases to trigger apoptosis. While caspase-8 and -9 function as initiator caspases, caspase-3, -6 and -7 act as effector caspases. Caspase-2 can function as both an initiator and effector caspase; although there are controversies regarding its role in DNA damage-induced apoptosis. It is believed that association of caspase-2 with p53-dependent death domain (PIDD) containing protein is important for activation of caspase-2. The objective of the present study is to determine whether caspase-2 and p53 are important for cisplatin-induced apoptosis.

Methods: 2008 ovarian cancer cells were treated with cisplatin to induce DNA damage. Protein expression of caspase-2,-3,-7,-8,-9, p53, and PARP were studied using Western blot. PARP cleavage was used to monitor cell death by apoptosis. Knockdown of caspase-2 was achieved by siRNA.

Results: Cisplatin treatment caused a concentration-dependent increase in the processing of procaspase-2 and cleavage of PARP. The levels of p53 increased with cisplatin treatment. Caspase-2 knockdown decreased cisplatin induced apoptosis. Caspase-2 depletion decreased processing of caspase-9, but did not appear to affect other Caspases. Cisplatin-induced upregulation of p53 was attenuated when caspase-2 was depleted. However, depletion of p53 attenuated but did not prevent cisplatin-induced cell death. Based on siRNA studies, knockdown of caspase-2 had more pronounced effect in reducing cisplatin-induced cell death than p53 knockdown.

Conclusions: Caspase-2 is involved in cisplatin-induced apoptosis in ovarian cancer 2008 cells. Activation of caspase-2 may occur via both p53-dependent and –independent pathways.

Sponsor: *N/A*

213 *(Oral)*

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REDUCED TOXICITY AND SELECTIVE UPTAKE OF PACLITAXEL VIA ENCAPSULATION IN RECONSTITUTED HIGH DENSITY LIPOPROTEIN

Purpose: Current barriers to cancer chemotherapy include i) toxic side effects, ii) the limited accessibility of the drugs to tumor tissue, and iii) multi-drug resistance developed by malignant tumors during treatment. Our laboratory has developed a novel drug delivery system utilizing reconstituted high density lipoprotein (rHDL) nanoparticles with reduced toxicity to normal tissues and selective receptor-mediated uptake of drugs. We demonstrate these advantages through maximum tolerated dose studies in mice and uptake mechanism studies.

Methods: The rHDL/paclitaxel (Ptx) nanoparticles were characterized with regard to size via transmission electron microscopy and cytotoxicity against cancer cells using the MTT assay. Maximum tolerated dose studies were performed in C57Bl/6 female mice comparing rHDL/Ptx with Taxol® and Abraxane®. The rHDL/Ptx nanoparticles were labeled with 125-Iodine and with 14C-paclitaxel to study the mechanism of Ptx uptake by PC3 cells. Incubations were carried out with rHDL/Ptx alone or rHDL/Ptx with a 10-fold excess of HDL3. The cells were processed to determine the internalization of rHDL/Ptx and selective paclitaxel uptake.

Results: The rHDL/Ptx nanoparticles were found to have a diameter of 11.4 +/- 3.1 nm and 5-20 fold enhanced toxicity against cancer cells when compared to free Ptx. Eighty two percent of the paclitaxel was taken into the cell through selective uptake apparently via the SR-B1 receptor. Incubation of the cells with HDL3, the natural ligand of SR-BI, decreased paclitaxel uptake to 30.6% as compared to rHDL/Ptx alone ($p<0.0001$). During studies with mice a 2.3-fold and 1.4-fold higher dosage of rHDL/Ptx could be administered with reduced toxicity compared to Taxol® and Abraxane®, respectively.

Conclusions: Conclusions Reconstituted high density lipoprotein (rHDL) resembles native HDL in size and shape and can encapsulate paclitaxel. Encapsulation of chemotherapy drugs in rHDL reduces the toxic side effects seen in other formulations of paclitaxel. The majority of the paclitaxel was taken up by selective uptake, presumably mediated by the SR -BI mechanism.

Sponsor: *N/A*

214 *(Poster)*

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ROLE OF C17ORF37/MGC14832 IN PROSTATE CANCER CELL PROLIFERATION AND MIGRATION

Purpose: C17orf37 is a hypothetical gene highly expressed in different forms of cancer and its overexpression is correlated with ERBB2 expression. In prostate cancer cells, C17orf37 expression is elevated compared to normal prostate epithelial cells. C17orf37 is expressed as a cytosolic protein in cancer cells as dense punctated spots. The protein predicts prenylation motif and confocal microscopy of GFP-fused C17orf37 protein in LNCaP prostate cancer cells show membrane localization. The purpose of this study is to understand the functional role of C17orf37 protein in prostate cancer cells.

Methods: Western immunoblot and RT-PCR were performed to detect C17orf37 levels in prostate cancer cells. By using C17orf37 specific siRNA, the gene was knocked down from DU-145 prostate cancer cells. In vitro tumor invasion assay system and agarose gel bead assay were used to study the effect of C17orf37 downregulation in invasive potential of prostate cancer cells. Messenger expression of various isoforms of VEGF and ERBB2 in siRNA treated prostate cancer cells were quantified using RT-PCR. Immunoprecipitation and colocalization performed to study C17orf37-annexin A2 interaction.

Results: siRNA mediated knock down of C17orf37 in DU-145 cells showed reduced migration in agarose bead assay. Interestingly, this knockdown resulted in a reduction of ERBB2 messenger expression which in turn downregulated two isoforms of VEGF. Using an in-vitro tumor invasion assay system we observed that C17orf37 downregulated DU-145 cells show reduced invasiveness compared to untreated or mock transfected cells. However, on overexpression of C17orf37 with transient transfection of GFP-fused C17orf37, an increased invasive potential was observed in DU-145 and PC-3 cells compared to mocktransfected cells with GFP plasmid only. To establish annexin A2 as an interactor of C17orf37, we could successfully pull down annexin A2 and C17orf37 by immunoprecipitation. Z-section confocal images of colocalization studies showed most of the C17orf37-anx A2 interaction in the perinuclear area of the cytosol.

Conclusions: C17orf37 is involved in prostate cancer migration which either directly or through ERBB2 regulates VEGF expression which in turn regulates invasiveness of cancer cells. Annexin A2 is a novel interactor of C17orf37. Understanding the functional significance of C17orf37-anxA2 interaction in prostate cancer cells, will help us to decipher the signaling pathways of C17orf37 protein involved in progression of prostate cancer.

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215 *(Poster)*

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SWI6 AFFECTS MUTAGENESIS BY REGULATING EXPRESSION OF POLYMERASE ZETA SUBUNIT REV7 IN SACCHAROMYCES CEREVISIAE

Purpose: People of all ages get cancer, nearly all types are more common in middle-aged and elderly people than in young people. Mutations are the molecular foundation of cancer and cancer does not happen in one second. Only after the mutations in cells accumulate to a certain level may cancer happen. Maintaining the stability of the genome is critical to cell survival and normal cell growth. Translesion DNA synthesis (TLS) is an important DNA damage tolerance system cells utilize to complete replication in the presence of DNA damage. DNA polymerase zeta, consisting of catalytic subunit Rev3 and regulatory subunit Rev7, is known to be involved in most spontaneous and damage-induced mutations in Saccharomyces cerevisiae. And human has the homologous Rev3 and Rev7. We did a systematic screen of the S. cerevisiae genome to look for novel genes required for DNA-damage induced mutagenesis. And we got Swi6 which is currently known as a transcription cofactor. We investigated if Swi6 affects mutagenesis by regulating the expression of current mutagenesis-related genes such as REV3, REV7, etc.

Methods: Forward and reverse mutation systems were used to determine the mutagenesis ;evel change after SWI6 is deleted in Saccharomyces cerevisiae. mRNA levels of REV3, REV7 and other known mutagenesis–related genes were examined after SWI6 deletion. Transformation of plasmid which can be induced to over-express Rev7 protein into SWI6 deleted strain was done and the mutagenesis level change was examined by the reverse mutation system.

Results: SWI6 deletion in Saccharomyces cerevisiae makes the cells show lower mutagenesis. Northern blot analysis shows that SWI6 deletion leads to the low level of REV7 mRNA indicating Swi6, as a transcription cofactor, is involved in the REV7 expression. Overexpression of Rev7 in SWI6 deleted cell makes the cell have an increased level of mutagenesis.

Conclusions: These studies demonstrate that Swi6, a transcription cofactor in Saccharomyces cerevisiae, is involved in affecting the cell mutagenesis by regulating expression of REV7, which is the regulatory subunit of Polymerase zeta. This will allow us to have a better understanding of how mutagenesis is regulated in the transcription level.

Sponsor: *N/A*

216 *(Poster)*

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REGULATION OF DNA POLYMERASE ETA IN SACCHAROMYCES CEREVISIAE

Purpose: : One inevitable consequence of Y-family polymerases ability to synthesize across DNA lesions is their overall reduced fidelity, even on undamaged templates. Therefore, a tight regulation of damage tolerant polymerases to prevent the accumulation of unwanted mutations due to their unrestrained activity is speculated. The present study was carried out to investigate the regulation of DNA Polymerase eta under normal conditions as well as after DNA damage induced by UV radiation in Saccharomyces cerevisiae. Pol eta when inactivated causes Xeroderma Pigmentosum-Variant (XP-V), a syndrome which predisposes humans to enhanced UV sensitivity and skin cancer

Methods: Northern blot analysis was carried out to look at the message . Western blot analysis was carried out to detect any changes in the protein amounts after UV damage. Cells were synchronized in G1 phase by using a yeast pheromone alpha-factor. Co-immunoprecipitation was carried out to detect any covalent modification of the protein. Survival assays and mutagenesis assays were carried out to study a UBZ mutant (had a point mutation in a domain responsible for ubiquitination).

Results: Our findings show that an increase in the message of RAD30 gene encoding Polymerase eta(Pol?) after UV treatment is not reflected at the protein level. Given this polymerase is of utmost importance in the S phase of the cell-cycle, no increase was found in the protein amounts in S phase after cells were treated with synchronizing agent. Co-IP results show that Pol eta is constitutively ubiquitinated and the mobility of protein on the gel confirms that the modification is mono-ubiquitination. A UBZ mutant which is defective in ubiquitination is more UV sensitive and shows higher mutation tendency.

Conclusions: We have discovered that degree of monoubiquitination shows cell cycle dependence and is also affected by UV damage. We have also found that rad30 allele defective in ubiquitination shows enhanced UV sensitivity and higher mutagenicity after UV damage. This study shows that ubiquitination of Pol eta is essential for survival of the cells after UV damage. Whether ubiquitination affects protein-protein interactions or regulates the localization of the protein at the site of damage are some of the possibilities which need to be explored.

Sponsor: *N/A*

300 *(Poster)*

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LEUKOCYTE-APROTININ ATRIAL FIBRILLATION STUDY (LAFFS)

Purpose: Atrial fibrillation remains the leading postoperative complication following cardiopulmonary bypass. A randomized trial was undertaken to evaluate the effectiveness of leukocyte filtration and aprotinin, applied separately and in combination, on the incidence of post-operative atrial fibrillation. Also studied was the impact of these adjunct interventions on post-surgical renal and neurological dysfunction.

Methods: A total of 1,220 patients undergoing primary isolated coronary artery bypass grafting were randomly assigned to one of four treatment groups. The control group (305 patients) received standard cardiopulmonary bypass with moderately hypothermic (34°C) cardioplegic arrest. In the filtration group (310 patients) leukocyte reducing filters were incorporated into the bypass circuit. The aprotinin group (285 patients) received full Hammersmith dose aprotinin. The combination therapy group (320 patients) received both aprotinin and leukocyte filtration.

Results: The incidences of atrial fibrillation were 25% in the control group, 16% in the filtration group, 19% in the aprotinin group and 10% in the combination therapy group ($P < 0.001$). Renal dysfunction was detected in 3% of the control group, 2% of the filtration group, 8% of the aprotinin group, and 5% of the combination group ($P < 0.005$). Neurological dysfunction occurred in 2% of the control group, 2% of the filtration group, 1% of the aprotinin group, and 2% of the combination group (P = n.s.).

Conclusions: Combination therapy with aprotinin and leukocyte filtration markedly reduced atrial fibrillation post-cardiopulmonary bypass, and was more effective than the individual treatments. Aprotinin treatment increased the incidence of renal dysfunction, and the addition of leukocyte filtration partially mitigated this detrimental effect of aprotinin.

Sponsor: *N/A*

301 *(Poster)*

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ASSESSMENT OF ENDOTHELIAL DYSFUNCTION IN MEXICAN AMERICAN CHILDREN AT HIGH RISK FOR THE DEVELOPMENT OF TYPE 2 DIABETES AND CORONARY HEART DISEASE. DREAMS 1.

Purpose: This study screened for endothelial dysfunction in overweight Mexican American children at risk for type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD).

Methods: The study subgroup was identified from a cohort (probands) of 5th grade school children (n=1065) of the Fort Worth Independent School district. Probands (n = 85) at risk for T2DM, and their siblings were evaluated by physical examination, lifestyle, family history, and fasting serum parameters. Two subgroups of children, high risk (HR) and low risk (LR), were further evaluated for ischemia induced endothelial dysfunction of the brachial artery by ultrasound assessment. HR children (n=11, age 11.6 ± 0.5 years) were selected based on the presence of acanthosis nigricans (AN), insulin>15u/ml, and TC/HDL-C ratio >4.0. LR children (n=11, age 12.4 ± 1.6 years) were selected based on being AN negative, insulin =15u/ml, and TC/HDL-C ratio = 4.0.

Results: By the nonparametric Mann-Whitney test, Doppler assessment of endothelial dysfunction showed no difference between the two groups with respect to change in diameter of the brachial artery following a 4-minute period of pressure induced ischemia. As expected by selection criteria, the HR when contrasted with LR, had increased levels of a number of the CHD serum parameters associated with increased risk for CHD and T2DM including increased signs of oxidative stress. Systolic blood pressure, weight, BMI, waist size, W/H ratio, TG, TC/HDLC, insulin, glucose, HOMA and a serum lipid oxidative parameter (dienes, trienes) were all significantly higher ($p<0.05$) in HR while HDL-C was lower with no difference in the inflammatory marker, C-reactive protein (CRP). However, the noninvasive ultrasound approach did not demonstrate endothelial dysfunction in the HR group. Spearman correlational analyses of brachial artery parameters indicated an absence in the post ischemia phase in the HR group of relationships of a number of parameters seen in the low risk group (LR) consistent with an altered metabolic state in the HR group.

Conclusions: These studies indicated that a number of the serum surrogate markers and antecedents of CHD including oxidative markers are present in the HR group of twelve year old children but the pathological processes associated with these serum markers has not yet measurably impacted the endothelium. Such results suggest that alteration of life style at this age may prevent or delay subsequent arterial. Supported by CDC (H75/CCH224064) & UNTHSC.

Sponsor: *CDC (H75/CCH224064) & UNTHSC.*

302 *(Poster)*

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THE REACTIVE OXYGEN SPECIES MEDIATE INTERMITTENT HYPOXIA CONDITIONING-INDUCED CARDIOPROTECTION

Purpose: Intermittent, normobaric hypoxia conditioning (IHC) protects canine myocardium from ischemia-reperfusion injury. We tested the hypotesis that reactive oxygen species (ROS) produced during cyclic hypoxia-reoxygenation evoke IHC-induced cardioprotection.

Methods: Dogs underwent IHC [5-8 cycles of 5-10 min hypoxia (9.5-10% FIO2) + 4 min normoxia], uninterrupted hypoxia conditioning (UHC) lacking intermittent normoxia, or consumed antioxidant N-acetylcysteine (NAC; 250mg/kg) 2 h before IHC, for 20 d. On day 21, the left anterior descending coronary artery was occluded for 1 h and reperfused for 5h. Infarct size (IS) and area at risk (AAR) were measured by standard dyeing technique; arrhythmias were detected by lead II ECG and scored.

Results: IHC was remarkably cardioprotective, but UHC failed to mitigate infarction or arrhythmias, and NAC blocked the protection. [Sham (n=6): 38±5.8 / 3.7±0.4; IHC (n=9): 1±0.3 (*) / 0.7±0.1 (*); UHC (n=4): 50±7.5 (†) / 4.5±0.5(†): IHC + NAC (n=4): 39±3.8(†) / 4.5±0.5 (†)] # Expressed as % IS/AAR / Arrhythmia score (mean ± SE; *P<0.001 v non-hypoxia sham; †P < 0.001 v IHC)

Conclusions: ROS produced during IHC sessions are essential for development of robust cardioprotection. (NIH support: HL-064785 and HL-071684)

Sponsor: *(NIH support: HL-064785 and HL-071684)*

303 *(Poster)*

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ROLE OF ETHNICITY AND GENDER IN THE RELATIONSHIP BETWEEN BODY FAT AND INFLAMMATORY RISK FACTORS IN THE NORTH TEXAS HEALTHY HEART STUDY

Purpose: Racial/ethnic minorities have greater rates of overweight/obesity than do whites. Significant differences in rates of overweight/obesity also exist between men and women. Increased adipose tissue is a source of inflammatory cytokine production that may contribute to obesity-related cardiovascular risk. Visceral adipose tissue (VAT) rather than subcutaneous adipose tissue is particularly associated with cytokine production. Therefore, the purpose of the study was to determine whether ethnicity and/or gender influenced relationships between body fat and inflammatory risk markers.

Methods: Body composition was evaluated in 89 subjects (29 males, 60 females; 28 Hispanic, 60 non-Hispanics) using body mass index (BMI), waist-to-hip ratio, and CT-determined L4L5 and total VAT. Blood samples were taken to measure cardiac C-reactive protein (CRP), fibrinogen, and interleukin-6 (IL-6).

Results: All measures of body composition were positively related with fibrinogen (p=0.01). BMI, L4L5 VAT, and total VAT were positively related with CRP (p=0.05). Only BMI was positively correlated with IL-6 (p=0.05). These relationships were further tested for interactions with gender and ethnicity. Associations between BMI and IL-6 with CRP were significantly modified by gender. Increases in BMI were associated with greater increases in CRP for men than for women (beta-coefficients=0.295 and 0.128, respectively, p=0.05). Conversely, there was a positive relationship between BMI and IL-6 in women (beta-coefficient=0.557, p=0.001), while the relationship was not significant in men. The association between BMI and CRP was significantly modified by ethnicity. There was a positive relationship between BMI and CRP in non-Hispanics (beta-coefficient = 0.232, p=0.001), while the relationship was not significant in Hispanics.

Conclusions: These results suggest that gender- and ethnicity-specific cut-off points in BMI may be needed to define risk based on inflammatory markers. However, relationships between VAT and inflammatory risk markers were not influenced by gender and ethnicity.

Sponsor: *NIH P20 MD001633*

304 *(Oral)*

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HIGH FREQUENCY VENTRICULAR ECTOPY CAN INCREASE SYMPATHETIC NEURAL ACTIVITY IN HUMANS

Purpose: The purpose of this study was to determine the effect of different rates of ventricular ectopy on sympathetic nerve activity in humans.

Methods: We measured muscle sympathetic nerve activity (SNA), coronary sinus catecholamine and arterial pressure during graded rates of ventricular ectopy (from 4:1 to 1:1, sinus to ectopic beat ratio) in a total of 21 patients referred for electrophysiologic testing.

Results: Both muscle SNA and coronary sinus norepinephrine increased significantly with increased ectopy frequency ($p < 0.05$). Moreover, the change in muscle SNA correlated significantly with the change in coronary sinus norepinephrine levels ($r = 0.72$, $p < 0.001$).

Conclusions: These data demonstrate that sympathoexcitation evoked by high rates of ventricular ectopy can contribute to a state of elevated SNA both in peripheral tissues and within the heart. This altered autonomic state may contribute to an increased susceptibility to life-threatening tachyarrhythmias in patients with high rates of ectopy.

Sponsor: *N/A*

305 *(Poster)*

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DELTA RECEPTOR PHENOTYPES AND VASCULAR CONDUCTANCE IN SKELETAL MUSCLE

Purpose: Obstructive vascular disease produces disabling leg pain, immobility and a spiral of declining muscle perfusion and function. Enkephalins appear to increase blood flow by reducing vasomotor transmission within sympathetic chain ganglia.

Methods: The current study was conducted to systematically test whether delta-1-receptor blockade would unmask an increase in delta-2-mediated hyperemia. ME (0.03ug/kg-30ug/kg) was pulsed into the distal, descending aorta in anesthetized dogs. Low dose BNTX (0.3 ug/kg) was administered and the ME was repeated.

Results: Aortic injection of methionine-enkephalin (ME) just proximal to the L5-ganglion produced an immediate increase in femoral vascular conductance that was preferentially blocked by the delta-2, opioid, antagonist, naltriben and much less effectively by the delta-1 antagonist, BNTX. Low dose BNTX consistently appeared to enhance the hyperemic effect of ME, suggesting an opposing delta-1 mediated increase in vascular resistance. After BNTX, the threshold was shifted left by one dose (0.1ug/kg) and the maximal effect was reduced by one third.

Conclusions: These data lend initial support for the hypothesis that enkephalins exert both dilatory and constrictor influences via interaction with opposing phenotypes of ganglionic delta-opioid-receptors.

Sponsor: *AHA TX Affiliate*

306 *(Poster)*

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HEART RATE VARIABILITY IS ALTERED IN RABBITS BY ACUTE FAT FEEDING

Purpose: The purpose of this study was to determine if acute overeating of a high-fat diet (HFD) altered heart rate variability (HRV) during the day and/or night.

Methods: Adult female New Zealand white rabbits (n=7) consumed a control diet (CONT) before switching to an ad-lib HFD. Using telemetry, 3-min ECG segments were recorded every 20 min for 3 d of CONT and the first 3 d of HFD. Daytime and nighttime data were analyzed for heart rate (HR), RR interval standard deviation (RRISD), RMS of successive RR interval differences (RMSSD), low frequency (LF) and high frequency power (HF), and LF/HF. HFD and respective CONT periods were compared by paired t-test with level of significance set at p=0.05.

Results: HRV was altered by HFD more at night than during the day. Nighttime HR increased 37% (174 ± 3.1 vs. 239 ± 1.1 bpm, p<0.05). Compared with CONT, nighttime RRISD, RMSSD and HF decreased 60% (30.9 ± 1.3 vs. 12.4 ± 0.4 ms, respectively), 66% (27.4 ± 1.2 vs. 9.3 ± 0.4 ms, respectively), and 86% (48.0 ± 3.7 vs. 7.2 ± 0.9ms2, respectively), (all p=0.05). Although nighttime LF decreased 67% (28.8 ± 2.4 vs. 9.6 ± 0.9 ms2), LF/HF increased 170% (2.0 ± 0.2 vs. 0.8 ± 0.1), (p=0.05). HFD-induced daytime changes showed similar trends, but significant decreases were noted only in RRISD (20.1 ± 0.8 vs. 15.5 ± 0.6 ms) and LF (17.5 ± 1.2 vs. 11.3 ± 0.8 ms2), (p=0.05).

Conclusions: Acute overeating of a HFD produced immediate nighttime HRV changes indicating parasympathetic withdrawal and a shift toward sympathetic dominance.

Sponsor: *NIH HL64913*

307 *(Poster)*

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EVIDENCE FOR POSITIVE FEEDBACK BETWEEN VAGAL TRANSMISSION AND DELTA-1-OPIOID RECEPTOR PHENOTYPES IN THE SINOATRIAL NODE

Purpose: The study was designed to test the hypothesis that the gradually increasing vagotonic activity during the short occlusions was itself the result of delta-1-receptor stimulation.

Methods: A microdialysis probe was placed in the canine SA node and perfused with saline. After equilibration, agents like BNTX and MEAP were introduced into the dialysate. The SA node artery was occluded and released five times at 10-minute intervals. Right vagus nerve was tested during the first, third and fifth occlusion.

Results: Vagal transmission was gradually improved during the occlusions. BNTX (selective delta-1-opioid receptor antagonist) blocked this improvement completely. The vagolytic response of MEAP was less effective following preconditioning protocol.

Conclusions: Improvement of vagal function during preconditioning is mediated via delta-1-opioid receptor. The loss of delta-2 response is probably due to preconditioning, however independent of delta-1-opioid receptor.

Sponsor: *AHA TX Affiliate*

308 *(Poster)*

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RELATIONSHIP BETWEEN INTERLEUKIN-6 AND CALCIUM SCORES - THE NORTH TEXAS HEALTHY HEART STUDY

Purpose: Inflammation is an important process in the development of cardiovascular disease. Thus, peripheral cytokines, such as interleukin-6 (IL-6), have been shown to play a crucial role in the development of atherosclerosis and can serve as markers for subclinical atherosclerosis. Latest research has shown the value of coronary artery calcium scoring and its ability to predict future cardiovascular events. This study assessed the association between IL-6 levels and coronary artery calcium deposits.

Methods: One hundred and thirty-two subjects were recruited from 11 clinics of the North Texas Primary Care Practice-Based Research Network. High sensitivity serum assays were used to measure IL-6 levels. Multi-slice computed tomography was used to calculate coronary calcium scores and the results were dichotomized as zero (no atherosclerosis) or greater than zero (atherosclerosis). Logistic regression was performed to calculate odds ratios (OR) and 95% confidence intervals (CI).

Results: Overall, 48 individuals (35.8%) had a calcium score of greater than zero. For every one-unit increase in the IL-6 level, the odds of an abnormal calcium score is 1.25 (OR: 1.25, 95% CI: 0.99, 1.57) After taking age, gender, smoking status, hypertension, diabetes, and cholesterol into account, a one-unit increase in the IL-6 level is associated with 1.16 greater odds of an abnormal calcium score (OR: 1.16, 95% CI: 0.90, 1.50).

Conclusions: These results strongly suggest an inflammatory process in the development of atherosclerosis.

Sponsor: *N/A*

309 *(Poster)*

Author: Leticia Gonzalez

Presenter: Leticia Gonzalez

Department: Integrative Physiology

Classification: GSBS Student

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PROENKEPHALIN DERIVED PEPTIDES IN CANINE NEUTROPHILS

Purpose: Opioids and neutrophils are proposed to interact in cardiac ischemic preconditioning and in ischemia reperfusion injury. Since neutrophils are potentially both source and target for the opioids, canine neutrophils were isolated and their enkephalin content was characterized by radioimmunoassay (RIA) utilizing antibodies specific for the C-terminus of Methionine-Enkephalin (ME) and ME-Arg-Phe (MEAP).

Methods: After culture,purified neutrophils and media were separated and protease activity was terminated in a boiling bath. Peptides were extracted and separated by size exclusion chromatography into large (e.g. proenkephalin), intermediate (e.g. peptide B and F) and small (e.g. ME and MEAP) fractions. The fractions were dried under vacuum, reconstituted in buffer and the enkephalins were determined by RIA.

Results: ME-assays primarily identified intermediate-sized peptides in cells and media with limited ME in the media and limited large precursors in the cells. The MEAP-assay identified primarily large and intermediate sized peptides in the cells. Very little MEAP was detected.

Conclusions: The absence of MEAP and the predominance of precursors suggest that enkephalin processing in neutrophils may differ from that in cardiomyocytes. The data further suggest that neutrophils could represent a substantive source of cardiac enkephalin during ischemia and reperfusion.

Sponsor: *NIH MORE program*

310 *(Poster)*

Author: Ana Chiapa

Presenter: Ana Chiapa

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DIMENSIONS OF SOCIAL SUPPORT AND THEIR RELATIONSHIP TO CORONARY ARTERY CALCIUM BURDEN

Purpose: To examine the relationship between social support, its different domains (emotional, informal, and instrumental support), and the presence of CAC.

Methods: This cross-sectional study consisted of 80 participants recruited from family medicine, internal medicine, and general medicine clinics in North Texas. The participants completed a questionnaire that included demographic measures, as well as social support measures developed by Ross (1989). Coronary calcium (CAC) was measured using computed tomography and was scored as a dichotomous variable: presence of calcification (Agatston score of > 0) versus no calcification (Agatston score of 0).

Results: Calcium scores ranged from 0 to 2394, with 45% of participants presenting with a calcium score greater than zero. After adjusting for cardiovascular risk factors (smoking, hyperlipidemia, hypertension, diabetes, BMI, and age), a one point increase in the total social support score resulted in a 42% reduction in odds of having CAC (OR = .58; 95% CI = .18-1.87); the same occurred for emotional support, with a 34% reduction in odds (OR = .66; 95% CI = 23-1.83). For informal health support, a one point increase resulted in a 48% decrease in odds of having CAC (OR = .45; 95% .16-1.32). Instrumental support had a minimal effect in the reduction in odds of CAC (OR = .92; 95% CI = .36-2.4).

Conclusions: Overall, our results show that higher levels of social support are associated with lower levels of CAC, independent of coronary risk factors.

Sponsor: *N/A*

311 *(Oral)*

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Presenter: R Brothers

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EFFECT OF PRAZOSIN ON THE CONTROL OF THE PERIPHERAL VASCULATURE DURING REST, LOW, MILD, AND HEAVY EXERCISE WORKLOADS

Purpose: Metabolic inhibition of alpha-1 mediated vasoconstriction in exercising muscles is evident in both animal and human models. A majority of the studies investigating the role of alpha-1 receptors have infused exogenous alpha1-receptor agonists intra-arterially to activate these receptors. However the role of physiological activation of these receptors in humans is incompletely understood. We aimed to test the hypothesis that as exercise intensity increases physiological activation of a1-receptors has a decreased role in the control of the peripheral vasculature in exercising muscles.

Methods: We compared the vasoconstrictor responses before and after oral ingestion of the alpha1-receptor blocker Prazosin (1mg/20kg of body weight) in healthy humans (n=7) during rest and dynamic knee-extensor exercise at roughly 40%, 60% and 80% of workloadmax. Ultrasound Doppler provided direct measurements of femoral blood flow (FBF) and intra-arterially measured blood pressures were used to calculate femoral vascular conductance (FVC).

Results: Prazosin resulted in significant increases FVC at rest when compared to the control protocol (+60 ± 6%). During exercise the Prazosin mediated increases in FVC decreased with increasing exercise intensities (+39 ± 4%, +25 ±4%, and +19 ± 6% respectively).

Conclusions: These data demonstrate that vasoconstriction elicited by physiological activation of alpha1-receptors with circulating endogenous agonists is maintained during "moderately high" intensity exercise. We conclude that probing the effects of alpha-1 mediated vasoconstriction with intra-arterial infusion of pharmacological agonists overestimates the "sympatholytic" effect of exercise.

Sponsor: *N/A*

312 *(Poster)*

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THE EFFECTS OF METOPROLOL ON TOTAL CARDIAC WORK DURING SUBMAXIMAL EXERCISE

Purpose: The purpose of this study was to determine percent change of myocardial oxygen consumption by calculating the triple product (TP) during exercise with ß1-AR blockade, and to determine whether the TP can be equalized by increasing the time of exercise of the ß1-AR inhibited individual.

Methods: Five male subjects (ages 23-28) performed two 30 minute bouts of submaximal cycling exercise at 50% and 75% VO2max with and without administration of metoprolol prior to exercise. Q was measured using an acetylene rebreathe technique, HR was monitored continuously via ECG, and systolic blood pressure (SBP) was measured during each cardiac output measurement. Cardiac work was estimated via calculation of the triple product of HR, SBP and SV.

Results: Resting TP with metoprolol (4.9 + 0.64) was not significantly different from rest (1.0 + .45, p=0.065). TP during 50% VO2max was significantly decreased with metoprolol, 62.3+7.9 and 43.2 + 3.3 (p=0.018, n=5). The average additional time calculated to equalize cardiac work during 50%VO2max exercise while on ß1-AR blockade was predicted to be 12.72 + 3.28 minutes. TP during 75% exercise with metoprolol was not significantly different from control, 81.6 + 3.5 and 84.1 + 5.8 respectively (p=0.816, n=3).

Conclusions: Administration of metoprolol during exercise decreases total cardiac work, however, the extent of the effect is largely determined by the change in stroke volume after the addition of metoprolol. We predict that by increasing the exercise time of the ß1-AR inhibited individuals, thereby increasing the total number of heart beats, would be a predicted method of equalizing the total cardiac work of the control exercise with exercise during ß1-AR blockade.

Sponsor: *N/A*

313 *(Poster)*

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THE EFFECT OF PHYSICAL FITNESS ON CARDIOVASCULAR VARIABILITY DURING ORTHOSTATIC STRESS IN ELDERLY

Purpose: Few studies tested if a better physical fitness prevents the age-related impairment of cardiovascular variability. This project compared heart rate variability (HRV) and systolic blood pressure variability (SPV) between 16 physically active (ACT) and 18 sedentary (SED) healthy elderly men and women during lower body negative pressure (LBNP).

Methods: Though group age was similar (ACT vs SED: 68.3±1.1 vs 67.5±1.5 yr), VO2peak was greater (32.1±1.9 vs 20.6±1.2 ml/kg/min, P<0.01) and BMI was lower in ACT than SED (25.0±1.0 vs 28.9±1.2, P<0.03). Baseline high-frequency (HF, 0.15 – 0.35 Hz) HRV was significantly greater in ACT (3.19±0.1.28 bpm2) than SED (0.30±0.06 bpm2), whereas low-frequency (LF, 0.07 – 0.15 Hz) SPV was not different between the groups (2.52±0.44 vs 3.50±2.22 mmHg2, P=0.68). Normalized HF (nHF) HRV at rest implied a vagal dominance in ACT (0.58±0.04) and a smaller vagal influence in SED (0.42±0.04).

Results: LBNP decreased nHF HRV (P = 0.04). Stratified by fitness factor, a significant correlation between LBNP and nHF HRV was found in ACT (P=0.01), not in SED (P=0.09). HF HRV was inversely correlated with HF SPV (P<0.01) and LF SPV (P<0.02) during LBNP.

Conclusions: A better physical fitness improved vagal cardiac function and helped buffer blood pressure variability during orthostatic stress. Our study implied that cardiovascular aging could be deferred by a physically active lifestyle.

Sponsor: *NIH grant HL65613*

314 *(Poster)*

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RELATIONSHIP OF RATE OF CALCIUM DEPOSITION IN THE CORONARY ARTERIES TO CARDIOVASCULAR DISEASE RISK FACTORS. DREAMS 2.

Purpose: To assess the relationship of Ca deposition in the coronary arteries to cardiovascular disease risk factors based both on rate of deposition and change in CVD risk factors. This is a pilot study using data of an ongoing study.

Methods: Three groups were compared for changes of Ca scores with varying degrees of Ca deposition in a one year period. In 12 months, Group A had Ca score change of 1-25; B had Ca score change of 25-100; and C had a Ca score change of >100 . We analyzed for differences between the 3 groups at baseline (V1) and a year later (V2).The three groups of subjects were free of evidence of CHD and were at NCEP-ATP III goals at time of enrollment. Electron beam computed tomography (EBCT) was used for coronary calcium (Ca) quantification at V1 and V2. Prevalence of traditional and emerging risk factors (ERF) were assessed.Statistical considerations: Means, medians, and standard deviations were calculated for continuous variables and ANOVA used to test for significant differences.

Results: Increasing Ca score trends were associated with changes in the traditional and emerging risk factors. ANOVA showed the most significant finding to be that the greater the Ca score, the lower the apoA-I concentration and these limited significant changes were primarily confined to V2. Analyses by change in levels from V1 to V2 showed changes ($p<0.10$) for SBP (in group A), waist circumference (C), apoA-I (A), apoB (C), ($p<0.05$)for SBP(C),TC(C), HDL-C(C),LDL-C (C), Lp(a) (A,B,C), homocysteine(B),insulin(C), A1c(A), and bilurubin(A),

Conclusions: Though limited by a small number of participants at this point, several deductions can be made. By trends, most factors associated with risk, change as Ca deposition in coronary arteries increases. Comparisons of the 3 stratified Ca accumulation groups showed little significant statistical difference with most noticed at V2 and primarily associated with lipid transport. Comparing changes in parameters from V1 to V2 showed a number of changes with Lp(a) changing for each Ca strata and with most significant changes associated with a Ca score change of >100. These studies point to the potential using CT Ca scoring to identify factors associated with atherosclerosis progression. Identification of these factors will serve as a basis for the application of more stringent primary prevention strategies in populations previously not considered at risk relative to their LDL-C.

Sponsor: *CDC (H75/CCH224064) & UNTHSC*

315 *(Poster)*

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RELATIONSHIP OF CARDIOVASCULAR DISEASE RISK FACTORS TO SITE OF CALCIUM DEPOSITION IN THE CORONARY ARTERIES. DREAMS 2.

Purpose: The usual site of greatest deposition of atherosclerotic lesions is the left anterior descending (LAD) coronary artery followed by the right coronary artery (RCA). We noted that in some individuals the RCA had greater Ca deposition than the LAD. The objective of this substudy was to assess the relationship of the site of Ca deposition in the coronary arteries to cardiovascular disease (CVD) risk factors with the hypothesis that the pattern of CVD risk factors would vary with site of Ca accumulation.

Methods: We compared changes of Ca localization in the RCA and LAD of three groups generated by taking the ratio of Ca deposition of RCA/LAD: RCA/LAD>1.0; RCA/LAD 0.05-1.0,and RCA/LAD <0.05. We analyzed for differences between the 3 groups at baseline. At the time of enrollment, the three groups of subjects were free of evidence of CHD. Electron beam computed tomography (EBCT) was used for coronary calcium (Ca) quantification and coronary artery localization. Prevalence of traditional and emerging risk factors (ERF) were assessed. Means and standard deviations for anthropomorphic variables and serum factors were calculated for each Ca localization group. One-way analysis of variance (ANOVA) was performed comparing the ERF factors among the three Ca localization groups.

Results: The site of localization of Ca in coronary arteries by groups showed a trend for a number of parameters where as the RCA/LDA increased, they also increased while others decreased. Those with a positive trend associated with an increasing ratio included age, SBP, BMI, TG, Hb1ac, TNF-a, MPO, leptin and the LMW dense LDL phenotype while both glucose and IL6 declined. These changes were not supported by ANOVA analyses which showed limited significant changes confined to PA1 ($p<0.05$) and leptin ($p<0.07$)

Conclusions: Though limited by a small number of participants at this point, several deductions can be made. By trends, a number of factors associated with risk, change as Ca deposition in RCA increases. Comparisons of the 3 Ca localization groupings showed little significant statistical difference. The finding of leptin related to advanced atherosclerosis was surprising and could be a reflection of other roles for leptin not currently recognized. A more careful match of the subjects could provide additional insights. These studies point to the potential of using EBCT determined Ca deposition in coronary arteries to identify factors associated with atherosclerosis progression in different vascular beds.

Sponsor: *CDC H75CCH224064 & UNTHSC*

317 *(Poster)*

Author: Ana Wilson

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ALTERED HEART RATE VARIABILITY IN RABBITS OVER A SIX WEEK PERIOD OF CONTINUED OVEREATING OF A HIGH FAT DIET

Purpose: We previously demonstrated that 3 d of acute overeating caused changes in heart rate variability (HRV) that were indicative of reduced parasympathetic tone. However, it is not certain whether detrimental changes in HRV further deteriorated with continued overeating. Therefore, this study determined if the acute overeating-induced changes in HRV were sustained during 6 weeks of continued overeating of a high-fat diet (HFD).

Methods: Adult female New Zealand white rabbits (n=3) consumed a control diet for 1 wk before changing to an ad lib HFD for 6 wks. Telemetry was used to record 3-min ECG segments every 20 min during the control week and the following 6 wks. Daytime and nighttime periods of inactivity were identified and data from these periods were used to determine low frequency power (LF), high frequency power (HF), LF/HF, RR interval standard deviation (RRISD), root mean square of successive RR interval differences (RMSSD), and heart rate (HR). The control period was compared with week 1 and with week 6 of HFD using paired t-tests with the level of significance at p=0.05.

Results: During the six week period body weight increased by 35.3% (3.43 ± 0.07 vs. 4.64 ± 0.07) as a result of ad lib HFD. Nighttime HRV was altered by HFD more significantly than daytime HRV. Nighttime HR and LF/HF increased the first week of HFD by 39.6% (182 ± 5 vs. 254 ± 10, p=0.05) and 166% (0.6 ± 0.2 vs. 1.6 ± 0.5), respectively. The change in nighttime HR was attenuated over 6 wks of HFD but remained 21.4% higher than the control value. The increased LF/HF ratio was sustained over 6 wks. LF, HF, RMSSD, and RRISD values decreased dramatically during the first week of HFD by 70.9% (14.1 ± 0.7 vs. 4.1 ± 1.6 ms2, p=0.05), 78.6% (22.9 ± 0.5 vs. 4.9 ± 1.9 ms2, p=0.05), 55.1% (22.5 ± 2.3 vs. 10.1 ± 3.9 ms, p=0.05), and 51.9% (25.5 ± 5.5 vs. 12.3 ± 2.4 ms), respectively. The acute reduction in HF was sustained over 6 wks.

Conclusions: Although the effects of the HFD on nighttime HR were attenuated over the 6-wk period, the acute overeating-related changes in nighttime HF and LF/HF were sustained over 6 wks of continued overeating and body weight gain. This suggests chronic parasympathetic withdrawal and a sustained shift towards sympathetic dominance.

Sponsor: *N/A*

318 *(Poster)*

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EFFECT OF ANGIOTENSIN II ON THE PERIPHERAL VASCULATURE DURING REST, LOW, MILD, AND HEAVY EXERCISE WORKLOADS

Purpose: To test the hypothesis that as exercise intensity increases AngII plays a greater role in the control of the peripheral vasculature.

Methods: We compared the vasoconstrictor responses before and after blockade of the Angiotensin type 1 receptor (AT1) with Valsartan (80mg) in healthy humans (n=5) during rest and knee-extensor exercise at ~40%, 60% and 80% of workmax. Neck suction (NS) stimuli were used to partially withdraw the arterial baroreflex mediated sympathetic activity. Ultrasound Doppler provided measurements of femoral blood flow (FBF) and blood pressures were used to calculate femoral vascular conductance (FVC).

Results: FVC was unchanged at rest and during the 40% and 60% exercise trials with Valsartan, however during 80% exercise FVC was increased by 17 ± 3% with Valsartan when compared to control exercise. NS elicited significant increases in FVC during rest and all 3 exercise trials. During 80% exercise NS mediated increases in FVC was further increased by 40 ± 7% with Valsartan when compared to control exercise.

Conclusions: These data identify that as exercise intensity and duration increases AngII plays a greater percentage role in the control of the peripheral vasculature.

Sponsor: *N/A*

319 *(Poster)*

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MURINE EXTRACELLULAR SUPEROXIDE DISMUTASE PHENOTYPE: ALLELE- OR STRAIN-SPECIFIC?

Purpose: Extracellular superoxide dismutase (ecSOD) is an antioxidant enzyme which protects the extracellular space from oxidative stress. We previously reported a novel polymorphism for ecSOD, expressed in 129P3/J (129) mice, which differs from the "wild-type, present in all other strains examined. It differs by a 10 bp deletion in the 3' UTR of the mRNA as well as two amino acid substitutions in the nascent protein. It is associated with a specific phenotype of dramatically increased circulating and heparin-releasable enzyme activity and mass. In order to examine the different properties of the two forms of ecSOD in an identical environment, we generated, by extensive backcrossing of ecSOD heterozygous progeny to C57 females, a congenic C57 strain with the 129 (or C57) ecSOD allele (C57.129-sod3). We found that by generation 6 (n6), the congenic mice achieved complete homozygosity with C57 for the six chosen markers, including one very near (14 cM) the ecSOD locus. Theoretically, n7 congenics have a 99.22% homology with the recipient (C57) strain. Genomic scanning revealed that n9 congenics have over 99% homology with each other.

Methods: In the present study we compare plasma pre- and post-heparin activities of ecSOD in n7, n9 and n10 congenics, bearing either allele for ecSOD, to the original parent strains of C57 and 129. Blood was collected into heparinized column by retro orbital plexus after anesthesia with or without hepain (100u) injection via the tail vein. Plasma enzyme mass was determined by Western blotting and ecSOD activity using a purely chemical system based on the oxidation of NAD(P)H.

Results: We could repeat the previous result that 129 has significantly increased free and heparin-releasable ecSOD activity and mass comparing those of C57 in present condition. Furthermore, we could examine both free and heparin-releasable ecSOD mass and activity in 129-sod3 mice are significantly higher than those in C57-sod3 mice in each generation.

Conclusions: Our finding suggests that a significant portion of the ecSOD phenotypic differences in the parent strains are directly due to the differences in the enzyme gene; a fraction of the observed phenotypic differences are attributable to overall strain differences in the environment in which the enzyme functions.

Sponsor: *RO1-HL70599*

320 *(Poster)*

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Department: Integrative Physiology
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INFLUENCE OF THE CAROTID BAROREFLEX ON CEREBRAL BLOOD FLOW DURING REST AND EXERCISE

Purpose: INTRODUCTION: Animal studies have shown that cerebral arteries are richly innervated with sympathetic nerve fibers. However, the role of autonomic neural control of the cerebral circulation remains controversial. Until recently it has been thought that changes in sympathetic tone have a limited effect on cerebral blood flow (CBF) at normal PaCO2 levels. PURPOSE: This investigation tested the hypothesis that sympathetic activation via the carotid baroreflex directly influences cerebral vasomotion at rest and during exercise.

Methods: METHODS: In five healthy human volunteers (mean ± SE: age 25 ± 2 yr ; height 166 ± 5 cm; weight 71 ± 7 kg) we examined the effects of pulsatile neck pressure (NP) and neck suction (NS) during rest and steady-state cycling exercise. Pulsatile (5-seconds on 5 seconds off; 0.1Hz) neck pressure (NP, +40 Torr) was applied for 6 minutes at rest and during exercise. Changes in heart rate (HR), mean arterial arterial pressure (MAP) and mean middle cerebral arterial velocity (MCA V mean), were measured at rest in the seated position and during leg cycling exercise with and without NP and NS.

Results: RESULTS: The power spectral density (PSD) of MAP at 0.1Hz increased during pulsatile NP and NS. Importantly, the PSD of MCA V mean at 0.1Hz was much greater during NP than that of NS at rest (4237 ± 1628 v 92 ± 101 cm2/s2/Hz, P = 0.015). Similar responses of PSD of MAP and MCA V mean to NP and NS was observed during dynamic exercise. There were no significant differences between end-tidal CO2 between each condition.

Conclusions: CONCLUSION: These findings suggest that cerebral vasoconstriction during NP was a result of the autoregulatory response to the NP mediated pulsatile changes in arterial pressure and the NP induced sympathetically mediated vasoconstriction.

Sponsor: *N/A*

319 *(Poster)*

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MURINE EXTRACELLULAR SUPEROXIDE DISMUTASE PHENOTYPE: ALLELE- OR STRAIN-SPECIFIC?

Purpose: Extracellular superoxide dismutase (ecSOD) is an antioxidant enzyme which protects the extracellular space from oxidative stress. We previously reported a novel polymorphism for ecSOD, expressed in 129P3/J (129) mice, which differs from the "wild-type, present in all other strains examined. It differs by a 10 bp deletion in the 3' UTR of the mRNA as well as two amino acid substitutions in the nascent protein. It is associated with a specific phenotype of dramatically increased circulating and heparin-releasable enzyme activity and mass. In order to examine the different properties of the two forms of ecSOD in an identical environment, we generated, by extensive backcrossing of ecSOD heterozygous progeny to C57 females, a congenic C57 strain with the 129 (or C57) ecSOD allele (C57.129-sod3). We found that by generation 6 (n6), the congenic mice achieved complete homozygosity with C57 for the six chosen markers, including one very near (14 cM) the ecSOD locus. Theoretically, n7 congenics have a 99.22% homology with the recipient (C57) strain. Genomic scanning revealed that n9 congenics have over 99% homology with each other.

Methods: In the present study we compare plasma pre- and post-heparin activities of ecSOD in n7, n9 and n10 congenics, bearing either allele for ecSOD, to the original parent strains of C57 and 129. Blood was collected into heparinized column by retro orbital plexus after anesthesia with or without hepain (100u) injection via the tail vein. Plasma enzyme mass was determined by Western blotting and ecSOD activity using a purely chemical system based on the oxidation of NAD(P)H.

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Conclusions: Our finding suggests that a significant portion of the ecSOD phenotypic differences in the parent strains are directly due to the differences in the enzyme gene; a fraction of the observed phenotypic differences are attributable to overall strain differences in the environment in which the enzyme functions.

Sponsor: *RO1-HL70599*

320 *(Poster)*

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INFLUENCE OF THE CAROTID BAROREFLEX ON CEREBRAL BLOOD FLOW DURING REST AND EXERCISE

Purpose: INTRODUCTION: Animal studies have shown that cerebral arteries are richly innervated with sympathetic nerve fibers. However, the role of autonomic neural control of the cerebral circulation remains controversial. Until recently it has been thought that changes in sympathetic tone have a limited effect on cerebral blood flow (CBF) at normal PaC02 levels. PURPOSE: This investigation tested the hypothesis that sympathetic activation via the carotid baroreflex directly influences cerebral vasomotion at rest and during exercise.

Methods: METHODS: In five healthy human volunteers (mean ± SE: age 25 ± 2 yr ; height 166 ± 5 cm; weight 71 ± 7 kg) we examined the effects of pulsatile neck pressure (NP) and neck suction (NS) during rest and steady-state cycling exercise. Pulsatile (5-seconds on 5 seconds off; 0.1Hz) neck pressure (NP, +40 Torr) was applied for 6 minutes at rest and during exercise. Changes in heart rate (HR), mean arterial arterial pressure (MAP) and mean middle cerebral arterial velocity (MCA V mean), were measured at rest in the seated position and during leg cycling exercise with and without NP and NS.

Results: RESULTS: The power spectral density (PSD) of MAP at 0.1Hz increased during pulsatile NP and NS. Importantly, the PSD of MCA V mean at 0.1Hz was much greater during NP than that of NS at rest (4237 ± 1628 v 92 ± 101 cm2/s2/Hz, P = 0.015). Similar responses of PSD of MAP and MCA V mean to NP and NS was observed during dynamic exercise. There were no significant differences between end-tidal C02 between each condition.

Conclusions: CONCLUSION: These findings suggest that cerebral vasoconstriction during NP was a result of the autoregulatory response to the NP mediated pulsatile changes in arterial pressure and the NP induced sympathetically mediated vasoconstriction.

Sponsor: *N/A*

321 *(Poster)*

Author: Devin Flaherty

Presenter: Devin Flaherty

Department: Integrative Physiology

Classification: Dual Degree Student DO/PhD

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THE ANAPLEUROTIC EFFECTS OF PYRUVATE DURING CARDIOPULMONARY BYPASS IN THE SWINE MODEL

Purpose: Cardiopulmonary bypass (CPB) is necessary for many complex cardiac surgeries including valve replacement, correction of congenital cardiac anomalies and difficult coronary artery revascularizations. Oxidative stress imposed on myocardium by cardioplegic arrest and reperfusion inactivates myocardial enzymes and produces cardiac edema. Pyruvate is a naturally occurring metabolic intermediate, energy substrate and powerful antioxidant. The antioxidant actions of pyruvate are proposed to interrupt systemic and intramyocardial inflammatory cascades, ameliorate injury to the myocardium and, thus, enhance post-surgical recovery of cardiac mechanical function as well as stabilize atrial rhythm.

Methods: In situ swine hearts are arrested via CPB for 60 minutes with either standard cardioplegia solution or cardioplegia solution augmented with pyruvate. The heart is then resuscitated and allowed a 4-hour recovery period. Upon completion of the recovery period, tissue samples from the left ventricle are freeze-clamped, and cardiac and pulmonary tissue samples are taken for enzyme activity assay via spectrophotometry. Throughout the protocol, plasma samples are periodically taken for pyruvate analysis.

Results: Current data from recent experiments (n=12; 4 pyruvate, 4 sham, 4 control) was processed and analyzed via spectrophotometry for CK, G6PDH. CK (Sham 28.87 +/- 2.2, Control 26.33 +/- 1.51, Pyruvate 60.16 +/- 15.88); G6PDH (Sham 0.009 +/- 0.003, Control 0.006 +/- 0.001, Pyruvate 0.008 +/- 0.001). Glutathione redox state, i.e. GSH/GSSG, was measured in arterial and coronary sinus plasma by HPLC and found to be significantly enhanced in the pyruvate-fortified group.

Conclusions: Pyruvate groups demonstrated an elevated redox state as compared to controls, as well as a significantly increased CK content. G6PDH was found to more closely approximate normal cardiac levels with pyruvate as compared to a lowered level under control conditions. Current aims focus on pyruvate, being a non-hypoxic stimulator of HIF-1 signaling pathway, potentially producing various protective proteins, such as erythropoietin (EPO), nitric oxide synthase (NOS), and heat shock proteins (HSP). Post-op inflammation and coagulation may also respond favorably to pyruvate treatment, and are being assessed in our model. Current analysis of cardiac and lung tissue for aquaporin channel activity during cardiopulmonary bypass is employing RT-PCR, immunohistochemistry and immunoblotting.

Sponsor: *N/A*

322 *(Poster)*

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Classification: Dual Degree Student MS/MPH

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BAROREFLEX MEDIATED VASOMOTION IS AUGMENTED DURING CENTRAL HYPOVOLEMIA: FREQUNCY DOMAIN ANALYSIS.

Purpose: Vasomotion oscillating around Meyer's wave (0.1 Hz) is augmented during unloading of arterial baroreceptors or cardiopulmonary baroreceptors, respectively. The question remained whether low frequency (LF) arterial blood pressure (ABP) variability was synergistically increased by activation of arterial baroreflex combined with central hypovolemia.

Methods: Five men and five women (28.9±1.8 years of age, 65.4±3.8 kg weight and 1.68±0.02 m height) voluntarily gave a written consent and participated in a study that was approved by IRB at UNT Health Science Center. Carotid arterial baroreceptor was selectively perturbed using 20 Torr of positive neck chamber pressure (PNCP) with 5 sec on-off oscillations for 6 minutes at rest and during -30 Torr of lower body negative pressure (LBNP). Heart rate interval (HRI, electrocardiogram), cardiac output (Q, Bio-Impedance), muscle sympathetic nerve activity (MSNA, Microneurography), systolic and diastolic ABP (SBP and DBP, Tonometry) were monitored.

Results: LBNP decreased Q by 18% (from 3.57±0.47 to 2.94±0.45 L/min, P =0.05) with no significant change in mean ABP (resting control vs LBNP: 80±2 vs 88±5 mmHg). LF (0.07 – 0.14 Hz) HRI variability from the combination of PNCP with LBNP was similar to the value from the addition of LBNP alone and PNCP alone. However, the addition of LF DBP variability was smaller (P =0.05) than the value from the combination of PNCP and LBNP. This vasomotion appeared to be independent of the response of MSNA variability since the difference in LF MSNA variabilities between the addition of PNCP alone and LBNP alone and the combination of PNCP with LBNP was not statistically significant.

Conclusions: We conclude that vasomotor oscillations are significantly augmented by the combination of unloading of carotid baroreceptor (by PNCP) with central hypovolemia (by LBNP) as compared to their simple addition. ABP variability (or instability) is exacerbated during unloading of both arterial and cardiopulmonary baroreceptors. [this study was supported by the NIH]

Sponsor: *N/A*

323 *(Poster)*

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CAM KINASE KINASE AS A PRIMARY DOWNSTREAM TARGET OF UROTENSIN II RECEPTOR IN SMOOTH MUSCLE CELLS

Purpose: Urotensin II (UII) plays an important role as an activator of vascular smooth muscle cell proliferation and hypertrophy, often contributing to vascular pathology when sustained and unabated. Urotensin II receptor (UIIR) signaling results in the activation of phospholipase C (PLC) and second messengers IP3/DAG, which trigger the release of calcium from the sarcoplasmic reticulum. We hypothesize that UIIR-coupled Gq signaling in pulmonary arterial smooth muscle cells (PAC1) activates CaM kinase kinase (CAM KK) as a primary downstream target.

Methods: We show here that UIIR signaling in PAC-1 cultures results in the immediate phosphorylation and activation of CaM KK. Activated CaM KK phosphorylates and activates CaM kinase I (CaM KI). Western blot analysis of CaMK1 using a combination with pharmacological inhibitors and UII stimulation, demonstrates increased phosphorylation.

Results: We report here that activated CaM KK/ CaM KI has at least 5 primary serine/threonine kinase cellular targets; P38, PKD, Akt, and Erk1/2. P38, PKD and/or Erk1/2 kinase activation results in the induction of the smooth muscle hypertrophy/embryonic gene program and activation of contractile marker genes. This program is transcriptionally driven by the activation of the above kinases recruits four primary transcription factors; Mef-2, ATF-2, GATA4, and SRF.

Conclusions: In summary, UIIR signaling in pulmonary arterial vascular smooth muscle cells, controlled through CaM KK stimulates calcium mediated stress activated kinases and hypertrophy induced smooth muscle cell embryonic gene programs.

Sponsor: *N/A*

324 *(Poster)*

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ETHNIC-SPECIFIC DIFFERENTIAL EFFECTS OF POLYMORPHISMS OF THE ATP1A2 GENE ON BLOOD PRESSURE

Purpose: This study aimed to study whether polymorphisms in the ATP1a2 gene, which codes for the Na+-K+-ATPase pump, influence BP and BP reactivity.

Methods: Genotyping: Genotyping was done in a sample of 63 normotensive Caucasian (C) and African American (AA)subjects. DNA samples were sequenced in an ABI PRISM® 3100 sequencer. The sequence was analyzed and aligned using Sequencing Analysis Software v5.2 and Sequencher™ v4.2. The PHASE software program was used to predict haplotypes and genotypes. Measurement of BP: Arterial BP was measured non-invasively using photoplethysmography at the finger. Physiologic experiments: 36 subjects consented to the physiologic studies. The experiments were performed in a semi-recumbent position. After voiding, subjects were instrumented for measurement of physiologic variables. Then, the subjects were given two kinds of pressor stimuli – hypoxic apnea (with 12%, 16%, and 21% O2) and cold pressor test (with 2°, 10°, and 18°C). A washout window of 5 and 15 minutes was used in between the bouts of hypoxic apnea and cold pressor stimuli, respectively. The order of the six experimental conditions was randomized for each subject. Statistical Analysis: Independent association of the dose of the ancestral (H1) haplotype of the ATP1a2 gene with baseline systolic and diastolic was examined separately in Cs and AAs by using stepwise multivariate linear clustered regression analysis using BMI; history of smoking, obesity, sleep apnea; and parental history of hypertension and type 2 diabetes as covariates. Association of the H1 haplotype with measures of the frequency domain was assessed using analysis of variance (ANOVA).

Results: Polymorphisms in the 3' UTR region strongly influence the baseline systolic as well as diastolic blood pressure levels in an ethnic-specific manner. Each additional copy of the ancestral H1 haplotype is associated with a significantly increased baseline blood pressure in Cs and a significantly reduced baseline blood pressure in AAs. The polymorphisms do not seem to influence inter-individual differences in response to pressor stimuli, the rate at which an individual's blood pressure returned to baseline after cessation of pressor stimuli or the blood pressure variability during application of a pressor stimulus.

Conclusions: Polymorphisms in the 3' UTR of the ATP1a2 gene can affect blood pressure homeostasis and may be an important determinant of personalized management of human hypertension.

Sponsor: *N/A*

400 *(Oral)*

Author: Sherry Sours-Brothers
Presenter: Sherry Sours-Brothers
Department: Integrative Physiology
Classification: GSBS Student

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TRPC1 IS INVOLVED IN CONTRACTILE FUNCTION OF GLOMERULAR MESANGIAL CELLS

Purpose: Contractility of mesangial cells (MCs) is tightly controlled by [Ca2+]i. Ca2+ influx across the plasma membrane constitutes a major component of mesangial responses to vasoconstrictors. TRPC1 (transient receptor potential cation channel, subfamily C, member 1) is a Ca2+ permeable cation channel in a variety of cell types. The present study was performed to investigate whether TRPC1 takes part in vasoconstrictor-induced mesangial contraction by mediating Ca2+ entry.

Methods: The contractile response of cultured MCs was measured in response to Angiotensin II (Ang II), with or without the knockdown of TRPC1 expression by RNA inhibition (RNAi). Glomerular filtration rate (GFR) and renal blood flow (RBF) were measured using inulin and para-aminohippuric acid (PAH) clearance in response to Ang II or endothelin-1 (ET-1). TRPC1 activity was blocked by infusion of an antibody specific for the pore region of the TRPC1 channel. Single channel activity was measured in cultured MCs by patch clamp technique, and changes in intracellular Ca2+ levels were measured using fura-2 dual-excitation wavelength fluorescent microscopy. Both sets of experiments were carried out in response to Ang II, with or without blockade of TRPC1 activity with antibody treatment or inhibition of TRPC1 expression by RNAi.

Results: We found that Ang II evoked remarkable contraction of the cultured MCs. Downregulation of TRPC1 using RNAi significantly attenuated the contractile response. Infusion of Ang II or ET-1 in rats caused a decrease in GFR. The GFR decline was significantly reduced by infusion of TRPC1 antibody that targets an extracelullar domain in the pore region of TRPC1 channel. However, the treatment of TRPC1 antibody did not affect the Ang II-induced vasopressing effect. Electrophysiological experiments revealed that functional or biological inhibition of TRPC1 significantly depressed Ang II-induced channel activation. Fura-2 fluorescence-indicated Ca2+ entry in response to Ang II stimulation was also dramatically inhibited by TRPC1 antibody and TRPC1 specific RNAi.

Conclusions: These results suggest that TRPC1 plays an important role in controlling contractile function of MCs. Mediation of Ca2+ entry might be the underlying mechanism for the TRPC1-associated MC contraction.

Sponsor: *National Kidney Foundation, American Heart Association*

401 *(Poster)*

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SINGLE MOLECULE DETECTION USING SURFACE PLASMON COUPLED EMISSION (SPCE) AND SILVER ISLAND FILMS (SIF)

Purpose: Our preliminary studies indicate that by limiting the thickness of the detection volume by Total Internal Reflection (TIR) and lateral dimensions by confocal detection, we can decrease the detection volume to ~7 attoL and detect 5-7 cross-bridges in muscle. Also, it is shown that adding oxygen scavenging reagents to the sample medium alleviates the problem somewhat, but does not eliminate it. We hypothesize that by combining the principles of Confocal TIR with Surface Plasmon Coupled Emission (SPCE) and by using silver island films, we can reduce the detection volume and increase brightness of fluorescence, which would significantly decrease fluorophore fluorescence bleaching.

Methods: Muscle contraction was studied in rabbit skeletal muscle. Muscle is fluorescently labeled and placed on a coverslip coated with a thin layer of noble metal (gold or silver). The laser beam when incident at a particular angle called the Surface Plasmon Resonance(SPR) angle penetrates the metal layer and illuminates the muscle. Also, skeletal myofibril lightly labeled with rhodamine-phalloidin was placed on glass, sapphire or Olympus coverslip coated with silver islands and observed through a confocal aperture.

Results: SPR angle is narrowly defined, therefore, only fraction of the light incident on a sample in TIRF illumination was able to penetrate the metal coating in SPCE. The dramatic dependence of fluorescence coupling of surface plasmons on the orientation of the molecule transition moment made this method useful in measurements of orientation changes. Also, the directional character of SPCE enables excellent suppression of unwanted noise. Coating of coverslips led to a significant decrease in photobleaching and also caused an increase in brightness because of increase of quantum yield of phaloidin, by the enhancement of local electromagnetic field or by decrease of quenching. The detection volume resulted in detection of 12 actin monomers.

Conclusions: The results make it clear that the long term objective to observe a single molecule of a contractile protein during contraction of muscle is feasible. The SPCE method will find application in experiments where data from large assemblies complicates interpretation. The results are also expected to answer questions regarding the mechanism of muscle contraction. This will hopefully provide better insight to a component of myosin-associated diseases, such as Familial Cardiomyopathies, muscle degeneration and muscle atrophy.

Sponsor: *N/A*

402 *(Poster)*

Author: JOHN TALENT
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SILVER NANOPARTICLES DECREASE PHOTOBLEACHING OF SKELETAL MUSCLE MYOFIBRILS ON METAL SURFACES

Purpose: The interaction between actin and myosin is best studied at the level of a single cross-bridge to avoid averaging over ensembles of molecules having different kinetics. It is preferable to study this interaction in working muscle to avoid possibility of different behavior of contractile proteins in solution versus those in whole muscle. This involves illuminating muscle in sub-femtoliter (10^{12} L, fL) detection volume with intense laser light, which leads to rapid photobleaching of the fluorophores that are labeling actin or myosin. We have recently shown that photobleaching can be reduced by exciting Localized Surface Plasmon polaritons (LSP) in metallic nanoparticles (Silver Island Films, SIF) adjacent to muscle myofibrils on high refractive index substrate.

Methods: We placed myofibrils on a glass substrate covered with a metal on which SIF nanoparticles had been deposited. We excited LSP by a direct illumination of SIF.

Results: The presence of the metal surface led to a significant enhancement of fluorescence and to a large decrease in fluorescent lifetime, much greater than that observed in the presence of SIF alone. Glass silver mirrors coated with SIF were the most effective. They decreased photobleaching by a factor of at least 2.5 making it feasible to measure signal of a single cross-bridge within a sarcomere.

Conclusions: We speculate that the effect was caused by greatly enhanced dipole-dipole coupling interactions between LSP's, which led to a significant increase of the spontaneous emission rate of the fluorophore. Field enhancement led to an increase of brightness, which effectively decreased photobleaching because it allowed illumination with a weaker laser beam. The decrease in fluorescence lifetime effectively decreased photobleaching because it minimized the probability of oxygen attack during the time a molecule was in the exited state.

Sponsor: *N/A*

403 *(Poster)*

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ATOMIC FORCE MICROSCOPY IN MUSCLE STUDY

Purpose: It has been reported that the binding of fluorescent phallotoxins to actin in thin filaments of skeletal myofibrils is not uniform [1]. Fluorescence microscope images show fast initial binding to both ends of the actin thin filament, in the Z-band and at the ends distal from the Z-band (pointed end). The fluorescence pattern becomes uniform only after several hours of incubation. The hypothesis is that phalloidin competes with the nebulin (one of the actin binding proteins) in binding to actin. It is expected that time dependent phalloidin binding along with nebulin unzipping can change structure of the sarcomere particularly in the Z band area. AFM (atomic force microscopy) imaging is used to identify morphological changes in skeletal muscle associated with phalloidin binding.

Methods: Myofibrils were prepared from rabbit psoas muscle [2] and incubated with Phalloidin (SIGMA) for 20 min. Microscopic samples were prepared from both "+" Phalloidin and "-" Phalloidin 0.05 mg/ml myofibrils smeared on microscopic slide and dried at room temperature. Imaging was performed on the AFM Explorer (ThermoMicroscopes/Veeco Instruments Inc.) in contact scanning mode with Non-Conductive Silicon Nitride Probe (Veeco Instruments Inc.). Size measurements and data analysis was processed with the Veeco SPMLab Version 6.0.2 software.

Results: AFM Images show the difference in size and shape phalloidin containing myofibrils vs. control myofibrils.

Conclusions: These differences primarily occurred in Z band confirming the previous findings about non-uniform binding phallotoxins to actin in muscle. It remains unknown whether or not unzipping nebulin changes the Z band morphology and whether those changes are permanent or disappearing after longer incubation with phalloidin. 1 Ao X, Lehrer SS. Phalloidin unzips nebulin from thin filaments in skeletal myofibrils. 1995 J Cell Sci. 108 (Pt 11):3397-403. 2 Borejdo, J., O. Assulin, T. Ando, and S. Putnam. 1982. Cross-bridge orientation in skeletal muscle measured by linear dichroism of an extrinsic chromophore. J. Mol. Biol. 158:391–414.

Sponsor: *N/A*

404 *(Poster)*

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Classification: GSBS Student

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MEF2C MEDIATES RETINOIC ACID-INDUCED CARDIOMYOCYTE DIFFERENTIATION

Purpose: To prevent the severe complications of stem cell replacement therapy for diseased myocardium, including neoplastic transformation and lack of tissue integration, cardiac progenitor/precursor cells (CPPC) must be programmed to differentiate and exhibit a cardiac phenotype prior to transplantation. In this study, we tested the effects of all-trans retinoic acid (atRA), a potent inducer of tissue differentiation, on cultured CPPC and identified myocyte enhancer factor 2C (Mef2C) as a putative transcriptional regulator of atRA-induced cardiomyogenic differentiation.

Methods: A well-established rat cell line of CPPC (h9c2 cells), cultured under reduced serum conditions, was exposed to atRA and characterized for mRNA and protein expression of cardiomyocyte-restricted biomarkers. Mef2C activity was determined using luciferase reporter assays and a dominant negative Mef2C construct. The activity of p38 MAPK, a known activator of Mef2C, was also determined using antibodies against phosphorylated p38 MAPK, pharmacological inhibitors, and dominant negative p38 MAPK plasmids.

Results: Treatment of h9c2 cells with atRA induced gradual increase in total cellular protein, morphological changes, phosphorylation of p38 MAPK, Mef2C-luciferase activity, and expression of cardiac myosin heavy chain (cMHC) and ventricular myosin light chain (MLC2v). Overexpression of dominant negative mutants of p38MAPK and Mef2C inhibited atRA-induced cMHC and MLC2V protein levels in a dose-dependent manner. Interestingly, atRA rapidly promoted sustained increases in Mef2C-luciferase activity without altering Mef2C protein levels suggesting involvement of non-classical transcription independent retinoid signaling.

Conclusions: In conclusion, our study shows that atRA promotes development of a cardiomyogenic phenotype in CPPC via p38MAPK and Mef2C signaling. Retinoic acid and other retinoids could prove useful in programming stem cells/CPPC to undergo cardiomyogenic differentiation prior to transplantation. In addition, our study identifies p38 MAPK and Mef2C as potential targets for pharmacological intervention in stem cell therapy. Last but not least, further investigations of direct activation of Mef2C by retinoids in cardiac stem cells could provide a critical initiation point for studies of molecular signals regulating cardiac differentiation and development of congenital heart defects.

Sponsor: *N/A*

405 *(Poster)*

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Presenter: Suzanne Gonzalez

Department: Cell Biology and Genetics

Classification: GSBS Student

Suzanne Gonzalez, UNTHSC John Planz, UNTHSC Michael Smith, UNTHSC Arthur Eisenberg, UNTHSC Margaret Garner, UNTHSC

SINGLE NUCLEOTIDE POLYMORPHISMS IN ATP1A2 GENE ASSOCIATES WITH DIABETES, METABOLIC SYNDROME, AND HYPERTENSION IN CAUCASIANS VERSUS CONTROLS

Purpose: The Sodium-Potassium-ATPase, a2 subunit (ATP1A2) is an integral membrane protein involved in establishing and maintaining the electrochemical gradients across the plasma membrane, which are essential for osmoregulation, sodium-coupled transport of molecules, and electrical excitability of nerve and muscle cells. Current studies suggest that ATP1A2 is a key regulator of blood pressure. Decreased ATP1A2 activity is associated with hypertension, obesity in via hyperphagia, and type 2 diabetes. Single Nucleotide Polymorphisms (SNPs) in the 5' and 3' untranslated region (UTR) of the ATP1A2 gene have been reportedly associated to hypertension, change of skeletal muscle glycolytic-to-oxidative enzyme activities ratio, and responsiveness to endurance training. Hypothesis: SNPs in the 5' and 3' UTR of the ATP1A2 gene are associated with diabetes, metabolic syndrome (MS), and hypertensive subjects compared to matched controls.

Methods: Medical histories were taken from 172 Caucasians and 64 African Americans. DNA from subjects was extracted from blood or buccal swabs. Highly polymorphic segments of the 5' and 3' UTR of the ATP1A2 gene were PCR amplified and sequenced. The resulting haplotype profiles and individual SNPs were analyzed for linkage disequilibrium via Hardy-Weinberg and genetic structure via AMOVA.

Results: Although no significant results were found within the African American population, a significant correlation between the individual SNPs relative to the populations among groups-affected (diabetes, MS, hypertension) vs. controls- was found in Caucasians (P-value<0.001). All reported SNPs were within Hardy-Weinberg equilibrium (HWE) in the control group; however, significant departures from HWE were found in the 5' SNPs 1 and 2 (P=0.016 and P=0.018) in the MS group, (P=0.008 and P=0.013, respectively) in the hypertensive group. Although all 3' SNPs were within HWE in all groups, SNP 6 approaches deviation in the MS group (P= 0.071).

Conclusions: The sample size in this study is too small to conclude significant associations between any of the reported SNPs and the diabetic, MS, and hypertensive subjects versus controls. However, a significant correlation between the individual SNPs relative to the populations among affective and non-affected groups was found in the Caucasian population (P-value<0.001). This in conjunction with individual deviations from HWE in MS and hypertensive groups warrants further study.

Sponsor: *N/A*

406 *(Poster)*

Author: Vaibhav Pawar
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CHECKPOINT ACTIVATION IN STATIONARY PHASE SACCHAROMYCES CEREVISIAE DEPENDS ON MITOCHONDRIAL RESPIRATION

Purpose: The purpose of our study is to understand how spontaneous endogenous DNA damage accumulating in non-dividing cells of Saccharomyces cerevisiae trigger checkpoint pathways.

Methods: To study non-dividing cells, we have grown the cultures from logarithmic phase to stationary phase. Activation of checkpoint pathways is analyzed by phosphorylation state of protein kinase Rad53 (the homolog of human CHEK2).

Results: Our experiments show Mec1 and Rad50 dependent Rad53 phosphorylation in stationary phase S. cerevisiae cultures within approximately 2 days of incubation if two major pathways of oxidative damage repair, base excision repair (BER) and nucleotide excision repair (NER), are inactivated. This effect is strictly dependent on mitochondrial function and therefore absent in rho0 strains (lacking detectable mitochondrial DNA). All single DNA repair pathway mutations tested were insufficient to induce Rad53 modification within this time frame. However, even the wild-type showed Rad53 phosphorylation if the incubation period in exhausted medium was drastically increased (> 3 months). Interestingly, a combination of YKU70 (encoding the budding yeast Ku70 homolog), but not of DNL4 (encoding DNA ligase IV), with defects in NER (rad1, rad4) or homologous recombination (rad52) elicited a rapid Rad53 phosphorylation response in stationary phase.

Conclusions: Multiple protection and repair mechanisms are in place to respond to oxidative stress in budding yeast therefore a combination of DNA repair pathways such as base excision repair (BER) and nucleotide excision repair (NER) has to be compromised to trigger Rad53 phosphorylation. The reason why rho+ strains (with functional mitochondria) show Rad53 phosphorylation unlike rho0 is may be due to mitochondrial respiratory activity. It has been shown that rho0 cells have reduced ROS as compared to rho+ cells. The importance of Rad50 in this system argues the presence of double strand breaks or aberrant telomeres as critical lesions. An absolute requirement of Mec1 for Rad53 phosphorylation shows involvement of strand break or telomere shortening type of phenotype. The known telomere alteration in yku70 mutant may synergize with an independent defect of oxidative damage repair in rad52 or in NER mutant to trigger Rad53 phosphorylation. These observations may relate to the premature aging phenotype of mice deleted for Ku.

Sponsor: *This study was supported by NIH grant ES11163*

407 *(Poster)*

Author: Shashank Bharill
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Shashank Bharill, Jeff Ballin, Ignacy Gryczynski, Gerald Wilson and Zygmunt Gryczynski

SITE SPECIFIC VARIATIONS IN RNA FOLDING THERMODYNAMICS

Purpose: To determine local transitions in the structure of RNA during folding by steady state and time resolved fluorescence spectroscopy of 2-Aminopurine (2-AP). This will provide a base to compare local structural transitions with global structural transitions and provide insight into the RNA folding processes.

Methods: The thermodynamic stability of RNA folding was assessed by thermal denaturation of samples containing 300 nM RNA,10 mM KHEPES/HOAc and either: [i] 50 mM KOAc and 5 mM Mg(OAc)2, or [ii] 4.5 M urea with or without 5 mM Mg(OAc)2. The thermodynamic stability of hairpin RNA folding was also estimated by equilibrium denaturation in urea. Fluorescence (370 nm) of 2-AP-labeled RNA samples (300 nM) was measured at 25 °C in 10 mM KHEPES/HOAc across a range of urea concentrations. These thermodynamic stabilities of RNA folding was measured both by Steady-state and Time-domain fluorescence methods.

Results: Thermal denaturation of RNA substrates was done and following normalization to the unstructured control, a 2-AP residue inserted in the bulged position (HP6) displayed a dramatic decrease in fluorescence with increasing temperature, exhibiting a well defined melting transition at 56 °C. 2-AP residues inserted into loop (HP14) and stem (HP21) positions also revealed structural transitions near 57 °C and 55 °C, respectively. Free energy of folding in absence of denaturant (?GUW) determined by chemical denaturation was greatest for the HP14 substrate, indicating that local RNA conformation within the loop position is more stable than near the bulged base or within the hybridized stem region. At 25 °C and 4.5 M urea, all 2-AP-substituted hairpin substrates exhibited distinct fluorescence changes as a function of Mg2+ concentration. Addition of Mg2+ induced the most dramatic increase in fluorescence emission from HP6, as the 2-AP base transitions from an unfolded and likely partially stacked state into the bulged conformation. 2-AP inserted into the loop region (HP14) yielded a much more modest enhancement of fluorescence, while emission from the stem-positioned 2-AP residue (HP21) was significantly decreased by Mg2+-dependent stabilization of the folded hairpin structure.

Conclusions: Our studies shows that using the above methods we can determine the localized transitions in RNA folding.

Sponsor: *N/A*

408 *(Poster)*

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Classification: Faculty

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CHARACTERIZATION OF TELOMERASE IMMORTALIZED CORNEAL EPITHELIAL CELLS

Purpose: To characterize CTECs by their protein profiles with particular emphasis on the expression of TAPs. To show that the telomerized cells are capable of DNA repair and differentiation in vitro

Methods: Primary human corneal epithelial cells were transfected with a retroviral plasmid for ectopic expression of telomerase. Expression of cell cycle proteins (P53 Rb, P63), cytokeratins, TAPs (Tin2, TRF and Tankyrase) and involucrin were determined by western blot analysis and indirect immunofluorescence. Response to adriamycin (0.2ug/ml) as a DNA damaging agent was followed over a period of 72 hours.

Results: Ectopic expression of telomerase results in immortalization of corneal epithelial cells. These cells express minimum levels of Tin2 and TRF proteins which are negative regulators of telomere elongation. They express high levels of Tankyrase which is known to be a positive regulator of telomere elongation. These cells express cytoskeletal proteins (keratins) and cell cycle proteins that are expressed by WT corneal epithelial cells. In response to DNA damage (adriamycin) there is activation of P53 mediated DNA repair pathway. This is demonstrated by the up regulation of P53 and corresponding increase in P21 in the presence of adriamycin. The immortalized cells are normally highly proliferative under normal culture conditions but they undergo differentiation in the presence of serum or high calcium containing medium. This is shown by the expression of differentiation markers involucrin and cytokeratin AE5.

Conclusions: We have shown that in corneal and conjunctival epithelial cells ectopic expression of telomerase extends proliferative in vitro life span. These cells express proteins similar to those expressed by the WT cells. When exposed to appropriate conditions these cells expresses increasing levels of differentiation markers and respond to DNA damage by activating the DNA repair pathway.

Sponsor: *N/A*

500 *(Poster)*

Author: Joan Carroll

Presenter: Joan Carroll

Department: Integrative Physiology

Classification: Faculty

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INSULIN RESISTANCE, METABOLIC RISK FACTORS, AND BODY COMPOSITION 6 MONTHS AFTER LAPAROSCOPIC GASTRIC BANDING SURGERY (DREAMS: DIABETES RESEARCH, EDUCATION, AND METABOLIC STUDIES)

Purpose: Purpose: The purposes of this study were to determine overall and gender-related effects of laparoscopic gastric banding (LGB) on body composition, insulin resistance, and metabolic risk factors.

Methods: Methods: 19 patients (8 men, 11 women) were evaluated at baseline (T1) and 6 months after LGB (T2) for body composition, blood pressure, metabolic risk factors, and Homeostasis model assessment-insulin resistance (HOMA-IR).

Results: Results: At T1, men had higher total visceral adipose mass than women (8.6±0.8 vs 5.2±0.5 kg, respectively, p=0.01), and tended to have a greater body weight and waist/hip ratio (p=0.10). HOMA-IR was 2.86±0.4 and 9 of 19 subjects had scores >2.5. At T2, all measures of body composition were significantly improved, although BMI was still 36.3±1.3 kg/m2. HOMA-IR declined to 1.33±0.2 and only 2 subjects had scores above 2.5. Reduced HOMA-IR was due to reduced fasting insulin with no change in fasting glucose. At T2, systolic blood pressure, cardiac CRP, and fasting fatty acids were reduced while ApoA1 was increased. Only the change in ApoA1 displayed gender differences, with women having greater increases than men (p=0.01). Plasma total and HDL cholesterol, and ApoB were not altered at T2.

Conclusions: Conclusions: There were significant improvements in risk factor profile 6 months after LGB, despite ongoing obesity. Improvements in most risk factors were not dependent on gender.

Sponsor: *CDC*

501 *(Poster)*

Author: Ximena Urrutia-Rojas

Presenter: Ximena Urrutia-Rojas

Department: School of Public Health

Classification: Faculty

*Urrutia-Rojas X, Menchaca J***, Willis, B, McConathy W, Jimenez M, Buttreddy, Sabitha, Marshall K, Bae S, Lacko, A., and Spellman C. UNTHSC Texas College of Osteopathic Medicine*, School of Public Health**, Graduate School of Biomedical Sciences**** and Cook's Children's Physician Network***, Fort Worth, Texas.*

THE PREVALENCE OF ABNORMAL GLUCOSE METABOLISM IN HISPANIC PARENTS OF CHILDREN WITH ACANTHOSIS NIGRICANS. DREAMS 1

Purpose: To assess the prevalence of abnormal glucose metabolism in Hispanic parents whose children are positive for acanthosis nigricans (AN). Our hypothesis is that parents with such children are at higher risk for diabetes.

Methods: As part of an ongoing project (DREAMS, Diabetes Research Education and Metabolic Studies program) involving the primary prevention of Type 2 diabetes (T2D) and cardiovascular diseases, 258 Hispanic families with overweight children were evaluated for metabolic and anthropometric parameters including fasting blood glucose levels, AN status, and prior history of T2D diagnosis. The criteria used were fasting impaired glucose (IFG), abnormal glucose (=126 mg/dl), or prior diagnosis of T2D.

Results: Of 343 parents screened at baseline(229 mothers and 114 fathers), a total of 92 (30.0%) of the parents whose children were AN+ (acanthosis nigricans positive) were noted to have an abnormality of carbohydrate metabolism. In contrast, only 17 (18.3%) of the parents (p=0.048) with AN- (acanthosis nigricans negative) children had abnormal findings. Among parents of AN+ children, 19.6% showed IFG, 5.6% with abnormal glucose,and 4.8% had a diagnosis of T2D. On the other hand, parents of AN- children,16.1% showed IFG, 2.2% abnormal glucose, and 0% had T2D. When mothers and fathers were considered separately, 17.3 % of the mothers with AN+ children had IFG levels compared to 7.1% with AN– children and 4.0% mothers with AN+ children had overtly elevated glucose compared to 1.8% with AN- children (p=0.028). Fathers of AN+ children showed a higher proportion of abnormal glucose levels compared to those with AN- children, but the difference was not significant.

Conclusions: Our findings suggest parents of Hispanic children with AN are at higher risk of carbohydrate metabolism abnormalities. It appears that mothers with AN+ children may be affected more frequently with abnormal glucose metabolism. Having a child with AN among Hispanics may need to be considered as an additional risk factor for focused screening of parents for T2D in this population. (Support: CDC H75/CCH224054 & UNTHSC).

Sponsor: *CDC H75CCH224064*

502 *(Poster)*

Author: Walter McConathy
Presenter: Sabitha Buttreddy
Department: Internal Medicine
Classification: Faculty

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IMPACT OF OVERWEIGHT AND TANNER STAGE ON DIABETES AND CVD RISK FACTORS

Purpose: The Tanner stages (TS) are related to physical development in children and adolescents as well as related to sex hormone changes. This study investigated the relationship of overweight (BMI > 25) to TS in Mexican American children with regard to biochemical and anthropomorphic parameters associated with metabolic syndrome (MS), Type 2 diabetes (T2D), and cardiovascular disease (CVD). Our objective was to assess the impact of overweight (BMI >25) on parameters associted with risk for Type 2 diabetes (T2D) and CVD at TS 1-5.

Methods: The DREAMS program involves the primary prevention of T2D and CVD. From 258 Hispanic families with overweight children, data regarding TS were evaluated for metabolic parameters related to carbohydrate (glucose, insulin, HOMA), CVD (TC, HDL-C, LDL-C, TG) obesity (leptin, CRP) and anthropometric parameters (BP, BMI, WC, W/H). Statistical considerations: Means and standard deviations were calculated for continuous variables. A 2 (BMI>25) x 5 (TS) factorial analysis of variance was performed using a general linear model (GLM) to ascertain changes in various anthropomorphic and serum parameters across TS and BMI>25 groupings.

Results: For most variables, a main effect was observed from TS and BMI > 25 status. Mean plots for a number of parameters, many related to metabolic syndrome are presented, e.g. systolic blood pressure (SBP). A few variables showed significant interaction between BMI> 25 and TS. In some cases this interaction occurred in one gender only. To further investigate this, simple main effects were tested for each TS and BMI 25 level to determine at which TS(s) that BMI> 25 had significant effect. An example of these comparisons is shown for the BUN/Creatinine ratio.

Conclusions: For almost each parameter, being overweight (BMI >25) in childhood was associated with increased risk for MS, T2DM, and CVD. These differences were evident in the early TS including prepubetal Stage 1 in both males and females while in some cases levels in Stage 4 & 5 were more similar. Interactions between BMI and TS with respect to levels of parameters were not evident except for BUN/creatinine ratio in females and in males, glucose and leptin. A larger cohort may have revealed statistically significant differences in a number of parameters not evident in the present study though the trends observed in this study point to an impact of overweight on T2DM and CVD risk beginning prepubetal.

Sponsor: *N/A*

503 *(Poster)*

Author: Shannon Bolinger
Presenter: Aggie Watson
Department: Physician Assistant Studies
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Shannon Bolinger, PA-S II, Fort Worth, Texas Aggie Watson, PA-S II, Fort Worth, Texas Patti Pagels, MPAS, PA-C, Fort Worth Texas Olive Chen, PhD, Fort Worth, Texas

SHOULD THE PRESENCE OF ACANTHOSIS NIGRICANS BE USED AS THE SOLE PREDICTOR OF INSULIN RESISTANCE IN THE ADOLESCENT POPULATION

Purpose: Despite conflicting research results regarding the use of Acanthosis Nigricans (AN) as the primary screening method, in 2003 the 77th Texas Legislature passed H.B. No. 2721 implementing the mandatory screening for AN in all public and private schools. The purpose of this study was to examine whether the presence of AN could be a reliable detector of an insulin resistant state in children ages 8-18.

Methods: This study was conducted as a retrospective chart review utilizing three clinics in the Dallas/Fort Worth metroplex. Intentional sampling was used based on the diagnosis code of AN. The inclusion criteria consisted of a child between the ages of 8-18, with a diagnosis of AN, and no previous diagnosis of diabetes. Data derived from the charts included: fasting serum insulin, fasting blood glucose, fasting lipid panel, HbA1c, C-peptide, and oral glucose tolerance test; pertinent patient demographics and family history was utilized for the study as well. SPSS 12.0 was used to perform ANOVA and Chi-Square.

Results: A total of 160 charts were reviewed in this study. The majority of the patients were female (57%). Hispanics (56%) and African Americans (35%) represented the two main ethnic groups. The average age of the study was 11.37. Of the patients diagnosed with AN, the data showed that the average Body Mass Index (BMI) was 31.31 (Obesity I); the most significant lab values were as follows: 1) Fasting insulin- 65% fell into the normal category, 30% fell into the high category; 2) Fasting Glucose- 95% fell into the normal category, 5% fell into the pre-diabetic category; 3) Fasting Cholesterol- 88% fell into the desirable category, 12% were borderline high; 4) HbA1c and Oral Glucose Tolerance test - 100% fell within normal range. The data also showed that BMI and fasting serum insulin levels reached a statistically significant positive correlation ($r = 0.421$, $p < 0.001$). The data demonstrated that among different ethnic groups, BMI values reached significant differences ($F = 3.772$, $p = 0.012$). African American patients fell into the Obesity II and Extreme Obesity categories while the Hispanic population fell into the categories of Overweight and Obesity I.

Conclusions: The results of this study support the finding that AN should not be used as the sole predictor for the development of DM-2, but should be used in conjunction with other identifiable risk factors such as an increased BMI, a positive family history of DM-2 and ethnicity.

Sponsor: *N/A*

504 *(Poster)*

Author: Walter McConathy

Presenter: Walter McConathy

Department: Internal Medicine

Classification: Faculty

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BODY MASS INDEX, BODY MASS INDEX PERCENTILE, AND WAIST CIRCUMFERENCE AS PREDICTORS OF RISK FOR DIABETES AND CARDIOVASCULAR DISEASE IN CHILDREN.

Purpose: In children and adolescents, body mass index percentile (BMI %) is commonly used to assess growth pattern and estimate body fat. Body mass index (BMI) and waist circumference (WC) are parameters used to assess overweight or obese status in adults. The goal is to compare BMI %, BMI and WC of children with respect to their relationship to a number of phenotypic markers related to Type 2 diabetes (T2D) and cardiovascular disease (CVD).

Methods: From 258 Hispanic families with overweight children (7-18years), data regarding BMI %, BMI and WC were evaluated for their relationships to metabolic parameters including carbohydrate (glucose, insulin, HOMA), CVD (TC, HDL-C, LDL-C, TG) and obesity (leptin, CRP) and anthropometric parameters (BP, W/H). Correlation coefficient and linear regression analyses were used to assess the relationships of BMI %, BMI and WC to these risk factors.

Results: Comparison of Pearson correlations of BMI %, BMI and WC for each gender with parameters related to carbohydrate, CVD, obesity and anthropometric parameters clearly demonstrated a stronger relationship of BMI and WC to a number of these parameters while BMI% correlations were less robust. Linear regression analyses to assess the strength of BMI %, BMI and WC in predicting levels several key parameters related to T2D (glucose, insulin) and CVD (HDL-C, TG, BP) showed the similarity of WC and BMI while BMI % was less effective.

Conclusions: BMI % represents a well accepted approach to assessing growth in children. Its utility in predicting risk is unclear though using cutoff percentile at the 85th and 95th is the usual criteria to assess obesity and its related risk. However, our preliminary analyses suggest that both BMI and WC are stronger predictors of levels of risk factors related to T2D and CVD than BMI %. These finding suggest that when assessing risk for T2D and CVD in children, BMI and WC provide a clearer assessment of risk than use of percentiles.

Sponsor: *CDC H75CCH224064*

505 *(Poster)*

Author: Brent Hawkins

Presenter: Sapril Nguyen

Department: Physician Assistant Studies

Classification: TCOM MPAS Student

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WHAT IS THE PREVALENCE OF TYPE 2 DIABETES RISK FACTORS IN A CLINICAL SAMPLE IN SOMOTO, NICARAGUA?

Purpose: In Nicaragua, there is a lack of prevalence data for those at risk for type 2 diabetes. The lack of data is more pronounced in rural areas of Nicaragua; therefore, the purpose of this study was to report the empirical data of type 2 diabetes risk factors in patients seen by the Manos Carinosas medical mission in June 2006.

Methods: This study was a retrospective chart review. Charts were from the Manos Carinosas medical mission trip to Somoto, Nicaragua in June 2006. The data extracted from the charts were risk factors for type 2 diabetes, which included Body Mass Index (BMI), blood pressure, waist circumference, and random capillary blood glucose (RCBG). These risk factors indicate the prevalence of risk factors for type 2 diabetes in those patients. The researchers then assessed the derived data: Patients with zero risk factors were assigned as "no risk", patients with one risk factor were assigned as "low risk", those with two risk factors were assigned as "moderate risk" and those with three risk factors were assigned as "high risk." Patients with four risk factors or above were categorized as "severe risk."

Results: Three hundred ten charts were reviewed. Of those 310 charts, only 186 met inclusion criteria. The majority of patients seen was female (84.9%) and at 18-39 years of age (51.1%). The data showed that 0.5% of the patients were assessed to have a severe risk for type 2 diabetes, 3.2% to have a high risk, 13.0% to have a moderate risk and 36.8% to have a low risk. Among all the charts reviewed, 53.5% demonstrated some risk for diabetes. Additional findings included that 20.2% met criteria for Impaired Fasting Glucose/Impaired Glucose Tolerance (IFG/IGT).

Conclusions: Greater than 53% of the patients seen by the Manos Carinosas mission in Somoto had at least one risk factor for type 2 diabetes. Another 20% had either IGT/IFT which was an additional finding to the original research question. These findings demonstrated the need for educational intervention to prevent type 2 diabetes in these patients in Nicaragua. The limitations and biases of this study included interrecorder reliability, the lack of discrimination between male and female waist circumferences, and a possibly skewed gender ratio. Healthcare providers should continue to screen for type 2 diabetic risk factors and to design a brief, effective educational program to further educate the patients about the disease and its medical consequences.

Sponsor: *N/A*

506 *(Poster)*

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Presenter: Sarah Ross
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Sarah E. Ross, BS; Susan F. Franks, PhD; Nicole Bereolos, MPH; Roberto Cardarelli, DO, MPH. Department of Family Medicine, University of North Texas Health Sciences Center, Ft Worth TX 76107

THE INFLUENCE OF COUNTRY OF ORIGIN ON GLYCEMIC CONTROL AMONG MEXICANS AND MEXICAN-AMERICANS WITH TYPE II DIABETES

Purpose: Diabetes mellitus remains a cause of significant morbidity and mortality among Hispanics/Latinos patients. Factors such as patient education, health beliefs, psychosocial factors, acculturation and overall lifestyle may positively or adversely affect glycemic control in patients with diabetes. Acculturation is the adoption of mainstream behavior, attitudes, and values. One of the major factors contributing to acculturation is the patient's primary developmental sociocultural environment (PSE) of their country of origin. Acculturation as it impacts various health conditions has been notably studied; however, no study has examined the influence of acculturation on diabetes control. The goal of this study is to determine the relationship between the PSE of country of origin and glycemic control among Hispanics/Latinos of Mexican ancestry with type 2 diabetes.

Methods: This observational cross-sectional study included 68 subjects. Hispanic/Latino adults of Mexican ancestry with a diagnosis of type 2 DM for at least one year were included. The instruments included a demographic questionnaire, a patient satisfaction questionnaire, the Generalized Acculturation Index and the Multidimensional Diabetes Questionnaire. Glycemic control was measured using the most recent Hemoglobin A1C (HbA1C) value from the medical chart.

Results: Primary developmental sociocultural environment of country of origin (PSE) was not significantly correlated with HbA1C (R=-0.6, p= 0.3). However, PSE and HbA1C each had in common significant associations with several key variables.

Conclusions: While primary developmental sociocultural environment of country of origin (PSE) was not directly significantly associated with HbA1C in this study, these two variables have in common significant associations with several other key variables. Significant correlations with HbA1C and PSE include the patient's frequency physician visits as well as other variables. These illustrate the potential connections that PSE may have in influencing glycemic control pathways associating country of origin with HbA1C. This study suggests that PSE as one aspect of acculturation influences glycemic control among Hispanic/Latino diabetic patients of Mexican ancestry through key aspects of psychosocial and behavioral influence. It is important for physicians to be aware of these issues when treating Hispanic/Latino patients.

Sponsor: *N/A*

600 *(Poster)*

Author: Barbara Adams

Presenter: John Bowling

Department: Family and Community Medicine

Classification: Faculty

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RURAL FAMILY MEDICINE TRACK FROM PAST TO PRESENT

Purpose: To summarize the 10 year history of the Rural Family Medicine Track and associated rural-based family medicine clerkship.

Methods: The Rural Family Medicine Track consists of various parts of the family medicine curriculum. Medical Students complete brief preceptorships at their assigned rural teaching site in Years 1 and 2, a family medicine core clerkship in Year 3 and the primary care partnership and geriatrics rotations in Year 4. Additional students have participated in the Year 3 rural-based core family medicine clerkship.

Results: The Rural Track and associated rural-based clerkship has been successfully implmented for ten years for a total of 160 TCOM graduates and 52 current medical students. Of the 52 students who indicated an overall post-experience rating for the Track, 69% rated it"excellent" and 27% rated it "good." Over 70% of graduates of the Rural Track selected primary care specialties for post-graduate training.

Conclusions: An average of 16% of TCOM students have participated in rural educational experiences from the Classes of 1998-2006. Those students who completed the Rural Track responded favorably to their experience and most rated it "excellent" or "good". Compared to TCOM graduates, a higher percentage of those graduates who participated in rural educational experiences entered primary care post-graduate training.

Sponsor: *N/A*

601 *(Poster)*

Author: Robert Kaman

Presenter: Robert Kaman

Department: Office of Outreach

Classification: Faculty

Robert L. Kaman, JD, PHD GSBS Outreach UNTHSC

OUTREACH PROGRAMS

Purpose: The office of Outreach administers programs whose principal goal is to increase the numbers of under-represented, disadvantaged or first generation college students entering the health professions and the biomedical sciences. The programs currently in place are the Adopt-A-School Program, SMART, McNAIR, More Knowledge In The Sciences (MKITS), The Go Center Project.

Methods: Each Program is distinct, but offers summer research internships for college (SMART, McNAIR), experiences in teaching science in K-12 (MKITS and SCORE), K-12 mentoring, tutoring and advising (Adopt-A-School, MKITS, SCORE, GO Center) and involvement of student organizations in a variety of activities (Adopt-A-School, MKITS, Go Center). Several partnerships with minority serving institutions have been developed and a student pipeline established between them and the various programs at the health science center.

Results: As a result of these efforts, the Office of Outreach has been recognized by Clinton and Bush White House Administrations for its success. The National Association of Outreach Admissions Professional named it the 1999 winner of its Excellence in Minority Admissions award and Minority Access, INC, has named the University of North Texas Health Science Center for the role model institution.

Conclusions: Efforts by the Office of Outreach have enabled the health science center to achieving great success in creating a diverse student population that leads the state in that area.

Sponsor: *N/A*

603 *(Poster)*

Author: Linda Reed

Presenter: Linda Reed

Department: Physician Assistant Studies

Classification: Faculty

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DETERMINING THE RELATIONSHIP BETWEEN MOTIVATION AND ACADEMIC OUTCOMES AMONG STUDENTS IN THE HEALTH PROFESSIONS

Purpose: The purpose of this study was to examine the relationship between learning motivation and academic outcomes for students in health professions programs.

Methods: The Modified Archer Health Professions Motivation Scale (MAHPMS) and a demographic survey were administered at orientation to 131 medical and 29 physician assistant students at the University of North Texas Health Science Center in the fall of 2005. At the end of the semester, the same version of the MAHPMS was administered, and final course grades and semester averages were collected. Descriptive statistics were analyzed for all the study variables. Analysis of variance was utilized to examine within subjects and between subjects differences for the learning motivation scores among programs and demographic categories. Linear regression analyses were used to determine the relationship between learning motivation scores and end-of-semester grades. And finally, logistic regression was performed to explore the ability of the motivation scores to predict academically high-risk students.

Results: Approximately three-fourths of the students indicated a preference for mastery learning (72.5%)and an internal locus of control (71.9%). For the PA students, alienation to learning (Pearson r = 0.637, R2 = 0.406, p = 0.001) and performance (Pearson r = 0.546, R2 = 0.319, p = 0.008) goal scores statistically related to semester grades, and alienation to learning scores predicted high-risk academic performance almost 90% of the time (p = 0.019). For the medical students, mastery goal scores statistically related to semester grades (Pearson r = 0.403, R2 = 0.162, p = 0.008), but no motivation score predicted high-risk performance to statistical significance. External locus of control scores predicted high-risk performance 81% of the time for the total group of students at the end of the semester (p = 0.008).

Conclusions: Students in this study exhibited learning motivation preferences similar to those of other health professions students reported in the literature. The findings of this study agreed with the literature on achievement motivation theory and raised questions regarding the effect of health professions curricula on student learning goals. Similar studies, measuring larger samples longitudinally need to be conducted in order to further validate or elucidate the results of this study.

Sponsor: *N/A*

604 *(Poster)*

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RURAL OSTEOPATHIC MEDICAL EDUCATION OF TEXAS: ROME-THE NEXT GENERATION

Purpose: To evaluate the initial semester of the Rural Osteopathic Medical Education of Texas (ROME) curriculum.

Methods: The ROME curriculum includes all the activities from the Rural Family Medicine Track as well as significant additional coursework and educational experiences designed to enhance training for students committed to a career in rural medicine. In addition to the preceptorships and clerkships of the Rural Track curriculum, the ROME training includes approximately 20-35 additional contact hours of classroom instruction per semester, unique rural experiences in Years 1 and 2, and a specially designed clinical rotation schedule during Years 3 and 4. Students evaluated each course component and the course as a whole.

Results: All 13 ROME students completed the Rural Medicine I course. Student comments regarding specific components of the curriculum were generally very positive. Their overall evaluation of the course reflected a satisfaction index of 94 (out of 100). Critique of the Rural Hospital Emergency Room Visit component indicated that students found this experience to be a valuable part of their education.

Conclusions: The Semester 1 ROME curriculum was well received by the students who participated. Students reported that they benefited from the focused rural medicine curriculum.

Sponsor: *N/A*

605 *(Poster)*

Author: Roberto Cardarelli

Presenter: Roberto Cardarelli

Department: Research

Classification: Faculty

Roberto Cardarelli, DO, MPH, FSSFP Elizabeth Palmarozzi, DO, Assistant Professor, Chairman, Department of Family & Community Medicine** Ana Luz Chiapa, MS* S. T. Coleridge, DO** *Universtiy of North Texas Health Science Center at Fort Worth, Department of Family & Community Medicine, Division of Education and Research, Fort Worth, Texas 76107 **Universtiy of North Texas Health Science Center at Fort Worth, Department of Family & Community Medicine, Fort Worth, Texas 76107*

PRIMARY CARE CLINICAL RESEARCH FELLOWSHIP

Purpose: The Primary Care Clinical Research Fellowship was developed within the Department of Family & Community Medicine and funded by a Health Resources and Services Administration grant (D56HP00170).

Methods: The fellowship offers a Master of Science in Clinical Research Degree through the Graduate School of Biomedical Sciences to be completed during the four year Doctor of Osteopathy curriculum. Also, fellows take an additional 31 hours of credit, complete two special problems, and a thesis project. Acceptance requires application, personal statement, and interview and is dependent on the likelihood of the medical student succeeding in the fellowship. Acceptance is based on transcripts, MCAT scores, research experience, desire, and interview outcome. The program selects two incoming medical students per year. Fellows receive a stipend to help offset the cost of graduate school courses as well as a small monetary award to help cover research costs (copies, supplies, etc.). Each is assigned an advisor/mentor and is required to have an approved degree plan early in their first year.

Results: Since 2003, 12 fellows have entered the program , 6 currently participate, one graduated in December 2006, and 5 left(3 in 2004, 2 in 2006). Fellows have published or have pending publication eight manuscripts, have participated in five National/State conference presentations with one 1st place award in a state medical student poster competition, and presented five posters at last year's Research Appreciation Day. Currently, five of the fellows are conducting health disparities-focused research.

Conclusions: Information about the program has been disseminated to departments, institutions, and extramural audiences. Internally, the Primary Care Research Journal and Division of Research website are informative. Extramural dissemination included a presentation at the June 2006 AACOM and 2007 Society of Teachers of Family Medicine National conferences. A manuscript about the program was published by the Osteopathic Medicine and Primary Care Journal. Additional promotion of the program during the initial TCOM application process is desirable. Retention may be addressed by a more rigorous applicant screening/interview process to identify those most highly motivated. Additionally, funds may be sought through other mechanisms to support costs, including the possibility of obtaining stipend funds for an "add-on" year devoted exclusively to clinical research.

Sponsor: *N/A*

606 *(Poster)*

Author: Roberto Cardarelli

Presenter: Roberto Cardarelli

Department: Research

Classification: Faculty

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EDUCATING PRIMARY CARE CLINICIANS ABOUT HEALTH DISPARITIES

Purpose: This article presents the evidence that disparities exist, how clinicians contribute to these disparities, and what primary care clinicians can do to reduce disparities in their practice.

Methods: It is important to educate primary care clinicians regarding this topic because they have the ability to have an impact in the reduction of health disparities.

Results: Clinicians are able to impact health disparities by receiving and providing cross-cultural education, communicating effectively with patients, and practicing evidence-based medicine. The changes suggested herein will have an impact on the current state of health of our nation.

Conclusions: Not Applicable

Sponsor: *N/A*

607 *(Poster)*

Author: Jasmeet Singh, Hunaid Gurji
Presenter: Jasmeet Singh, Hunaid Gurji
Department: Texas College of Osteopathic Medicine
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ATTITUDES AND PERCEPTIONS OF FIRST YEAR OSTEOPATHIC MEDICAL STUDENTS ON RESEARCH, EDUCATION, AND POSTGRADUATE TRAINING

Purpose: The purpose of this study is to understand the perceptions and attitudes of first year medical students on research (basic or clinical) and the impact the decisions have on their postgraduate training.

Methods: A 22-item survey measuring attitudes and perceptions of first year osteopathic medical students about research (basic or clinical) was administered on paper with a response rate of 79.5% (n=128). Items were scored using a 5 point Likert scale (strongly agree, agree, neutral, disagree, strongly disagree). Top two box with bottom two box analysis was used for each question. The neutral responses were left out in order to view committed responses. SPSS v14 was used for statistical analysis (frequency, t-test) with 95% confidence. Demographic data was collected for age, gender, and highest degree obtained.

Results: The demographics were as follows: gender 45% (53) males, 54% (64) females; age 21-49 years (mean=25); highest degree 77% (85) Bachelors, 22% (24) Masters, and one (<1%) with a doctorate. Among the students 89.9% (115) strongly agreed or agreed (SaA) with the statement that, "research was important to the practice of medicine" ($p<0.001$). However, 15.6% (20) SaA with the statement "I plan on pursuing a medical career with a main focus in research related clinical medicine" ($p<0.001$). In regards to performing research, 47.7% (61) SaA that "no additional degree beyond D.O. was necessary to perform research" ($p<0.001$), however the number of responses increased to 92.1% (117) SaA with the statement having a dual degree increases your likelihood of conducting research ($p<0.001$). Approximately 81.3% (104) of students SaA that "the length of time was a factor" in their decision to complete an additional degree (Masters or Doctorate) ($p<0.002$); however when asked if "financial incentive was more important than the length of additional training", 54.3% (70) SaA with this statement ($p<0.002$).

Conclusions: A majority of the students feel that research is important in their future clinical practice; although, their involvement in research was not a primary concern. Additionally, most students felt that their D.O. was sufficient to allow them to conduct research. When forced to assume that they must obtain an additional degree, an overwhelming majority of students believed that the length of the training program was a more significant factor than financial aspects.

Sponsor: *N/A*

700 *(Poster)*

Author: Ming-Hui Yang
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AQUAPORIN-9 CHANGES IN EXPRESSION UPON INSULTS AND UNIQUE LOCALIZATION TO MITOCHONDRIA IN RETINAL GANGLION CELLS

Purpose: The water channel, aquaporin9 (AQP9) is enriched in selected neuronal populations and is unique in its ability to act as a lactate-glycerol channel supplying neurons with alternative fuel under ischemic conditions especially due to its expression in the mitochondria. Therefore, its expression may alter under various insults inflicted on retinal ganglion cells (RGCs). The purpose of the study was to examine AQP9 expression in RGCs under conditions of elevated intraocular pressure (IOP) and following intravitreal injection of endothelin.

Methods: Insults on retinal ganglion cells were induced by elevation of intraocular pressure in rat eyes or via intravitreal injection of endothelin. Immunohistochemistry using a combination of thy-1/aquaporin-9 antibodies was used to follow changes in the retinal ganglion cell layer. Real-time PCR was used for measuring changes in AQP9 and beta-actin. Also, changes in AQP9 were followed in total retinal extracts using Western blotting and in vitro following hypoxic conditions with isolated RGC-5 cells.

Results: Intravitreal injection of ET resulted in reduction of AQP9 as determined by RT-PCR after 48hr. Similar reduction was observed in total retinal extracts after 72hrs. By contrast, optic nerve head astrocytes showed enormous up-regulation of AQP9 that co-localized with GFAP. However, changes in AQP9 in the Morrison model rats gave contradictory changes showing increases or decreases in retinas of different animal as determined by RT-PCR. Such changes did reflect different IOP differences between animals. Interestingly, AQP9 was localized to mitochondria in RGC-5 cells and its expression increased upon insults such as hypoxia.

Conclusions: Changes in AQP9 expression may be correlated with extent of cellular injuries in retinal ganglion cells suggesting a novel role in retinal ganglion cellular death upon elevation of intraocular pressure.

Sponsor: *N/A*

701 *(Poster)*

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Classification: GSBS Student
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REGULATION OF CALCIUM INFLUX IN RETINAL GANGLION CELLS BY SIGMA-1 RECEPTOR LIGANDS AND NEUROPROTECTION RELEVANCE

Purpose: Compared to our knowledge of more conventional receptor systems, the study of Sigma receptors is still in its infancy. Therefore, the purpose of this study is to investigate the effect of sigma-1 receptor ligands on the intracellular calcium levels and its role in neuroprotection.

Methods: Western blot and reverse transcription polymerase chain reaction (RT-PCR) were used to analyze the expression of sigma-1 receptor in normal and sigma-1 receptor overexpressing RGC-5 cells. The calcium imaging was used to measure the intracellular calcium levels changes after glutamate or KCl stimulation. Co-immunoprecipitation was used to determine any interaction between sigma-1 receptor and voltage gated L-type calcium channel. The whole-cell patch clamp was used to measure calcium currents in rat cultured retinal ganglion cells with or without sigma-1 receptor ligands. The calcein-AM / propidium iodide cell survival assay was used to study the neuroprotective effect of sigma-1 receptor ligands on glutamate-induced excitotoxicity.

Results: Western Blot and RT-PCR showed that sigma-1 receptor are expressed retinal ganglion cells. The calcium imaging results have shown that sigma-1 receptor ligand, (+)-N-allylnormetazocine hydrochloride [(+)-SKF10047] differentially inhibits the glutamate and KCl induced calcium influx in both types of RGC-5 cells while BD10047 reversed the effect of (+)-SKF10047 in RGC-5 cells. KCl induced calcium influx was affected by depletion of calcium from the perfusion buffer. Co-immunoprecipitation was used to show for the first time the association between L-type calcium channels and sigma-1 receptors. Whole-cell patch clamp of rat cultured retinal ganglion cells and hippocampal neurons demonstrated that sigma-1 receptor ligand (+)-SKF10047 inhibits L-type voltage gated calcium channels current. The calcein-AM/propidium iodide cell survival assay showed that sigma-1 receptor ligand (+)-SKF10047 protect RGC-5 cells from glutamate-induced excitotoxicity.

Conclusions: These results suggest that increase in intracellular calcium leads to the activation of sigma-1 receptor, which inhibit of NMDA receptor and voltage gated L-type calcium channels. Inhibitions of calcium influx by sigma-1 receptor ligands protect RGC-5 cell from glutamate-induced excitotoxicity. Protection of retinal ganglion cells from glutamate-induced excitotoxicity by sigma-1 receptor ligands may lead to the development of a new series of neuroprotective agents for the eye.

Sponsor: *ARP*

702 *(Poster)*

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Presenter: Raghu Krishnamoorthy
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Classification: Faculty
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ENDOTHELIN-1 UPREGULATES ENDOTHELIN B RECEPTORS TO PROMOTE APOPTOSIS OF RAT RETINAL GANGLION CELLS

Purpose: Endothelin-1 (ET-1) administration has been shown to produce optic nerve axonal loss and apoptosis of retinal ganglion cells, similar to that seen in glaucoma. However, the receptors through which ET-1 mediates these effects are not clearly known. The purpose of this study was to determine if ETB receptor activation contributes to cell death of retinal ganglion cells both in culture and in vivo in rats.

Methods: Wild-type and ETB-deficient rats were intravitreally injected with 2 nmole ET-1, sacrificed 48 hr post-injection and retina sections analyzed for apoptotic changes by TUNEL. In a separate set of experiments, ETB expression was analyzed by immunohistochemistry, in retinas of wild-type rats injected with ET-1. To study some of the signaling mechanisms contributing to ET-1 mediated cell death, virally transformed rat retinal ganglion cells (RGC-5 cells) were treated with 100 nM ET-1 for 24 hr and analyzed for phosphorylation of c-Jun N-terminal kinase (p-JNK). Additional tests to assess apoptotic changes were carried out by analyzing cytochrome c release into cytosol in RGC-5 cells treated with ET-1 for 24 hr. Apoptotic changes were also assessed by flow cytometry in RGC-5 cells treated with ET-1 in the presence or absence of endothelin receptor antagonists.

Results: Intravitreal ET-1 treatment produced an appreciable increase in apoptotic cell death of retinal ganglion cells in wild-type rats. The ET-1 mediated increase in retinal ganglion cell death was attenuated in ETB-deficient rats. ET-1 mediated retinal ganglion cell death in wild-type rats was accompanied by increased expression of ETB receptors, particularly in the retinal ganglion cells. Measurements by flow cytometry showed that ET-1 mediated apoptosis of RGC-5 cells in culture, which was also blocked by pretreatment with the ETB receptor antagonist, BQ788. ET-1 (100 nM) treatment of RGC-5 cells for 24 hr produced an increase in phosphorylation of c-JNK and also promoted cytochrome c release into the cytosol, indicative of apoptotic changes mediated possibly through mitochondrial pathways.

Conclusions: Elevations in ocular endothelin concentrations (as seen in primary open angle glaucoma) produce increased ETB receptor expression, and contribute to apoptosis of retinal ganglion cells. These studies suggest the possibility of developing endothelin receptor antagonists as potential neuroprotective agents in attenuating retinal ganglion cell death seen in glaucoma.

Sponsor: *N/A*

703 *(Poster)*

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RETINAL PIGMENT EPITHELIUM MODULATES RETINAL CELL DIFFERENTIATION: A POSSIBLE ROLE IN RETINAL STEM CELL NICHE

Purpose: Growth factors secreted by rat retinal pigment epithelial (RPE) cells including basic fibroblast growth factor (FGF-2), nerve growth factor (NGF), and pigment epithelium-derived factor (PEDF) were tested for their influence on differentiation and proliferation of retinal precursor (661W) cells.

Methods: The cellular response to growth factors was monitored by a proliferation bioassay and phase contrast microscopy. In addition, cell viability was measured by Calcein AM and ethidium homodimer incorporation. Markers for mature (opsin, glial fibrillary acidic protein) and immature markers (nestin, Pax6) were evaluated by fluorescent microscopy. The 661W cells were also treated with RPE-secreted proteins (RPE-SP) alone or depleted of NGF by immunoprecipitation.

Results: The 661W cells grown in serum expressed high levels of opsin (a marker for rod photoreceptor cells), but not glial fibrillary acidic protein (GFAP) (a glial cell marker), nestin (an early neuroepithlelial cell marker), or Pax6 (a transcription factor that controls retinal progenitor cell differentiation). 661W cells treated with FGF-2 exhibited a multiple-process morphology with small phase-bright cell bodies similar to neurons, while cells cultured in NGF or RPE-SP displayed rounded profiles devoid of processes. In sharp contrast, PEDF caused 661W cell proliferation and cell process formation. After 3 days, 661W cells grown in FGF-2 were slightly increased, but not significantly above control levels; cells treated with RPE-SP or NGF were 63% and 49% of control levels. 661W cells treated with RPE-SP immunodepleted of NGF were diminished to 61% of cell numbers measured in cultures grown in total RPE-SP. All treatment conditions resulted in almost 100% viability based on Calcein AM staining, but insignificant cell death as determined by ethidium homodimer incorporation. Further, FGF-2 caused an up-regulation of opsin protein expression by 661W cells.

Conclusions: In conclusion, 661W precursor cells proliferated and appeared to mature morphologically in response to FGF-2. In contrast, RPE-derived NGF and purified NGF inhibited proliferation and morphological differentiation of 661W cells, possibly inducing cell cycle arrest. These findings suggest that the RPE regulates the retinal stem cell niche and retinal cell differentiation via growth factors including FGF-2 and NGF.

Sponsor: *N/A*

704 *(Poster)*

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ENDOTHELIN RECEPTOR, ETA & ETB EXPRESSION AND ET-1 MEDIATED EXTRA CELLULAR MATRIX - COLLAGEN REGULATION IN HUMAN LAMINA CRIBROSA CELLS.

Purpose: Endothelin-1 (ET-1) has been implicated in glaucoma pathology. ET-1 mediates its functions through G-protein coupled seven transmembrane receptors, ETA and ETB. A primary site of injury in POAG appears to be at the level of lamina cribrosa (LC) which demonstrates extensive extra cellular matrix (ECM) remodeling. The purpose of this study was to determine the expression of endothelin receptors and ET-1 effects on the regulation of ECM in Lamina cribrosa cells.

Methods: Expression of preproET-1, a primary gene transcript of ET-1, ETA and ETB receptors in human lamina cribrosa cells (LC cells) was determined by RT-PCR. ET-1 mediated intra-cellular calcium changes and NO released in culture media, in the presence of 1, 10 & 100nM ET-1, 1μM BQ788 an ETB antagonist and 1?M BQ123 an ETA antagonist was measured using Fura-2 calcium imaging and Griess colorimetric assay respectively. ET-1 mediated regulation of ETA, ETB, collagen I and collagen VI in LC cells in the presence of 1, 10 & 100nM ET-1 was determined by QPCR and Western blot. Expression of collagen I and collagen VI in LC cells following ET-1 treatment was also determined by immunocytochemistry.

Results: ET-1 increased intra-cellular calcium concentrations in a dose-dependent manner which was blocked by BQ610, an ETA antagonist and not by BQ788 an ETB antagonist. ET-1 mediated a dose-dependent increase in NO release which was blocked by BQ788 and as well as BQ610. Desensitization to ETA - mediated increase in intracellular calcium was observed in LC cells following pre-treatment with ET-1 for 24 hrs and a corresponding decrease in ETA receptor expression was observed. ET-1 mediated an increase in ETB receptor, collagen I and collagen VI expression.

Conclusions: Human lamina cribrosa cells express functional ETA and ETB receptors and their expression and function can be altered in response to prolong exposure to ET-1. In LC cells ET-1 also regulates extra cellular matrix –collagen synthesis. The effects on ECM may be important in POAG subjects who have elevated plasma and aqueous humor levels of endothelin-1.

Sponsor: *N/A*

705 *(Oral)*

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REGULATION OF CYTOSOLIC CALCIUM LEVELS BY INTRACELLULAR CALCIUM CHANNELS IN ROD BIPOLAR CELLS.

Purpose: In neurons, there are two sources of Ca2+ involved in initiating Ca2+-dependent processes within the cytosol, Ca2+ entry from the extracellular space on the plasma membrane via voltage- and/or ligand-gated Ca2+ channels and Ca2+ release from intracellular stores such as the endoplasmic reticulum via inositol 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptor (RyR). Previous studies have identified the expression of intracellular Ca2+ channels in mouse retinal neurons but localization and functional studies are needed to fully understand the role of these intracellular receptors in different processes such as neurotransmitter release. The present study analyzed the localization of the different isoforms of IP3Rs and RyRs, and determines their influence on cytosolic Ca2+ concentration in mouse rod bipolar cells. We hypothesize that rod bipolar cells express different isoforms of IP3Rs and RyRs, and these intracellular Ca2+ channels are involved in Ca2+ signaling within these neurons.

Methods: Murine retinal neurons were acutely dissociated. Immunocytochemistry studies were performed on rod bipolar cells to determine the expression of RyRs and IP3Rs, and their localization. The cytosolic Ca2+ concentration of acutely isolated rod bipolar cells was monitored optically with microspectrofluorimetry and in the presence of modulators of intracellular Ca2+ channels.

Results: Immunocytochemical analysis of IP3R types 1, 2, and 3 and RyR types 1, 2, and 3 shows that mouse rod bipolar cells express different isoforms of IP3Rs and RyRs in region specific orientations. Activation of IP3R by its agonist IP3-AM produced an increase in intracellular Ca2+ levels which was abolished by pretreatment with the IP3R antagonist, Xestospongin D. Similarly, increases in cytosolic Ca2+ were seen when RyRs were activated with the RyR agonist, caffeine. Ca2+ transients were decreased by pretreatment with the RyR antagonist, ryanodine.

Conclusions: Results suggest that intracellular Ca2+ channels are involved in Ca2+ signaling of rod bipolar cells and can potentially function as pharmacological targets in neurodegenerative retinal diseases with Ca2+ dyshomeostasis.

Sponsor: *N/A*

706 *(Poster)*

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THE NEURAL ACTIVITY-REGULATED GENE HOMER 1A PROTECTS RETINAL NEURONS AGAINST GLUTAMATE-INDUCED CELL DEATH

Purpose: Homer 1a is an immediate early gene that can be selectively upregulated by neural stimulation. It has been shown that Homer complexes contribute to receptor surface expression, receptor clustering, and mGluR coupling to ion channels. In this study we investigated the possible role of Homer 1a in glutamate-induced cell death of retinal neurons.

Methods: Wild type and Homer 1a knockout model systems were used in our studies. Intravitreal injection with glutamate was used to induce cell death of retinal neurons. Histological analysis was performed 7 days post-injection. The TUNEL assay was used measure numbers of the apoptotic cells in retinal ganglion cell layer. Also, organotypic retinal cultures were used in our ex vivo studies. Cultured wild type or Homer 1a knockout P13 retinas were treated with glutamate. Apoptosis in the ganglion cell layer was examined with the TUNEL assay.

Results: Microscopic analysis revealed significantly more TUNEL-positive cells in the ganglion cell layer in Homer 1a knockouts with glutamate injection than in wild type or control injected groups. Apoptosis in the ganglion cell layer of cultured retinas showed dose dependent glutamate toxicity with more apoptotic cells in Homer 1a knockout retinas after glutamate treatment compared to wild type retinas.

Conclusions: The present study provides evidence for a potentially protective role of Homer 1a in glutamate induced cell death in the retinal ganglion cell layer.

Sponsor: *NEI*

707 *(Poster)*

Author: Tara Tovar

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Classification: GSBS Student

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TISSUE TRANSGLUTAMINASE EXPRESSION AND ACTIVITY IN NORMAL AND GLAUCOMATOUS TRABECULAR MESHWORK CELLS

Purpose: Glaucoma is a leading cause of irreversible blindness in the world. A major risk factor for glaucoma is elevated intraocular pressure due to increased resistance of aqueous humor outflow through the trabecular meshwork (TM). In the glaucomatous TM there is an increased accumulation of extracellular matrix (ECM) material due to a disruption of the normal balance between ECM deposition and degradation. Tissue transglutaminase (tTgase) belongs to a family of calcium-dependent enzymes that catalyze the post-translational modification of the ECM by cross-linking proteins, thus making these proteins resistant to enzymatic and physical degradation. It is possible that the increase in ECM proteins seen in the glaucomatous TM is due to increased cross-linking activity of tTgase. The purpose of this study was to determine if there are differences in expression and activity of tTgase between normal and glaucoma TM cells.

Methods: Normal (N=3 NTM) and glaucomatous (N=3 GTM) human TM cell lines were grown until confluent and cell lysates were collected using an extraction buffer. Western blot analysis was used to compare tTgase protein levels in NTM and GTM cells. tTgase enzyme activity between NTM and GTM cells was studied using a biotin cadaverine assay. In addition, immunohistochemistry was utilized to evaluate the expression of tTgase, fibronectin (FN) and N epsilon gamma glutamly lysine protein in NTM and GTM tissues.

Results: Western blot analysis and immunohistochemistry demonstrated the presence of tTgase protein in both NTM and GTM cells. There was a significant increase in tTgase protein in GTM cells compared to NTM cells. In addition GTM cells demonstrated a significant increase in tTgase enzyme activity compared to NTM cells. Immunohistochemical results demonstrated increased expression of tTgase and FN in GTM tissues. Immunohistochemistry also demonstrated an increased co-localization of FN and N epsilon gamma glutamyl lysine protein indicating significant cross-linking of FN by tTgase in GTM tissues.

Conclusions: This study demonstrated that both NTM and GTM cells express tTG. In addition, tTgase protein levels and enzyme activities are significantly elevated in GTM cells. There was a significant increase in co-localization of FN and N epsilon gamma glutamyl lysine protein in GTM tissues. These results indicate that tTgase may play an important role in the pathogenesis of glaucomatous by cross-linking ECM proteins such as FN and thus making the ECM more resistant to degradation.

Sponsor: *Alcon Research, LTD. and NSF Project SCORE grants*

708 *(Poster)*

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ROLE OF ANNEXIN A2/TISSUE PLASMINOGEN ACTIVATOR/PLASMINOGEN COMPLEX IN RETINAL GANGLION CELL DEATH

Purpose: Apoptosis of retinal ganglion cells (RGCs) is a characteristic trait of glaucoma. Elevated intraocular pressure induces ischemic damage with associated excessive glutamate release and calcium influx, triggering a cascade of apoptotic events. The downstream events leading to RGC damage after excitotoxic injury are poorly understood. Recent evidence suggests that RGCs respond to excitotoxic injury by activating protease-mediated pathological remodeling of the extracellular matrix through the plasminogen activator (PA)-plasminogen (PG) system and associated matrix metalloproteinases. Annexin A2 is a calcium binding protein which serves as a fibrinolytic center at the cell surface by recruiting matrix remodeling proteases and their substrates such as collagen 1, tissue plasminogen activator (tPA), plasminogen (PG), procathepsin B and tenascin C. The purpose of this study is to determine the role of the annexin A2 fibrinolytic center and its associated proteases in extracellular matrix breakdown and subsequent detachment induced apoptosis of retinal ganglion cells

Methods: Expression of annexin A2 was demonstrated in the rat retinal sections by immunohistochemistry and in RGC-5 cells by Western blot analysis, RT PCR and immunocytochemistry. Immunofluorescence staining followed by confocal microscopy was performed to colocalize annexin A2 and tPA on the surface of RGC-5 cells. Membrane bound annexin A2 and tPA were isolated using a calcium chelating buffer. Changes in the protein levels of annexin A2 and tPA on glutamate toxicity induction were assessed by western blot analysis. Lipofectamine-based transfections were used knockdown the intracellular and cell surface levels of annexin A2

Results: Annexin A2 is abundantly expressed in the ganglion cell layer and it binds to the extracellular surface of RGC-5 cells in a calcium dependent manner. Annexin A2 and tPA were shown to be co-localized on the surface of RGC-5 cells.After RGC-5 cells have been challenged with glutamate, changes in intracellular and cell surface protein levels of annexin A2 and tPA are observed. Modulation of intracellular and cell surface levels of annexin A2 is possible upon transient transfection with pDrive-sh AnxA2, offering a means to inhibit activity of the fibrinolytic center on the extracellular surface.

Conclusions: Our studies are directed towards understanding the role of annexin A2-associated extracellular proteases in retinal ganglion cell death and offering strategies for neuroprotection

Sponsor: *Research supported by the National Institutes of Health (CA109593 and MD 001633)*

709 *(Poster)*

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EXPRESSION OF 14-3-3 ISOFORMS IN THE HUMAN CORNEA

Purpose: To determine the expression of the 14-3-3 proteins in the human cornea, the conjunctiva and the primary cells comprising these tissues.

Methods: Using immunofluorescence, we determined the expression of 14-3-3 isoforms sigma,eta,theta,gamma,beta,epsilon and zeta in paraffin sections of the human cornea and conjunctiva. Using indirect immunoflouresence and western blot analysis we also determined the expression of these isoforms in primary corneal and conjunctival epithelial cells, keratocytes and endothelial cells. Furthermore, expression in primary epithelial cells was compared with that in several human corneal and conjunctival cell lines. Western blot analysis was used to confirm the presence of 14-3-3 isoforms in the culture medium from corneal epithelial cells and cell lines.

Results: All the 14-3-3 isoforms were expressed in the corneal and conjunctival epithelia and the primary epithelial cells and cell lines. Expression of 14-3-3 sigma was confined to epithelial cells and was secreted into the culture medium of the primary cells and cell lines.

Conclusions: We have determined that all the mammalian 14-3-3 isoforms are expressed in the human cornea, conjunctiva and the component cells, and that 14-3-3 sigma and gamma isoforms were found to be secreted into epithelial cell culture medium. We propose that the intracellular and extracellular presence of 14-3-3sigma supports its involvement in the epithelial specific signaling pathways.

Sponsor: *N/A*

710 *(Oral)*

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Department: Cell Biology and Genetics

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BONE MORPHOGENETIC PROTEIN 4 INHIBITS TGF-B2 SIGNALING IN OPTIC NERVE HEAD ASTROCYTES AND LAMINA CRIBROSA CELLS: EXTRACELLULAR MATRIX MODULATION BY GREMLIN IN GLAUCOMA

Purpose: The characteristic cupping of the optic nerve head (ONH) in glaucoma is associated with elevated TGF-ß2 expression and altered synthesis and deposition of extracellular matrix (ECM) proteins. Bone morphogenetic proteins (BMP) have been reported to selectively inhibit the fibrotic action of TGF-ß2. We hypothesize that in glaucoma, elevated TGF-ß2 levels in the ONH induce expression of gremlin, a secreted BMP antagonist, which selectively blocks BMP antagonism of TGF-ß2 signaling. This antagonism would result in increased ECM synthesis and deposition. The purpose of this initial study was to demonstrate that A) TGF-ß2, BMP-4, fibronectin, and gremlin is localized in human ONH, B) TGF-ß2 treatment upregulates gremlin expression, and C) gremlin inhibition of BMP-4 increases TGF-ß2- stimulated fibronectin (FN) production in cultured human ONH astrocytes and LC cells.

Methods: Immunohistochemistry was used to examine TGF-ß2, BMP-4, fibronectin, and gremlin protein level in age matched normal and glaucomatous ONH tissues (N=3). Human ONH astrocytes (N=4) and LC cells (N=5) were utilized. Effect of TGF-ß2 (5ng/ml) on gremlin expression was examined by QPCR and western blot analysis. To examine BMP-4 inhibition of TGF-ß2 signaling, ONH astrocytes and LC cells were treated exogenously with a) TGF-ß2 (5ng/ml) or BMP-4 (10ng/ml), b) TGF-ß2 and BMP-4, c) TGF-ß2, BMP-4 and gremlin (300ng/ml), and fibronectin content was assessed by western blot.

Results: Immunohistochemistry analysis demonstrated that protein for TGF-ß2, fibronectin, and gremlin is increased in glaucomatous ONH tissues. BMP-4 is localized in ONH and does not seem to be increased in glaucomatous ONH. Exogenous TGF-ß2 treatment increases gremlin mRNA and protein secretion in conditioned media of ONH astrocytes and LC cells. Western blot analysis demonstrated that TGF-ß2 increases fibronectin secretion significantly and that exogenous BMP-4 does not have significant effect on fibronectin synthesis and secretion. Coincubation of BMP-4 and TGF-ß2 reduced TGF-ß2 stimulated synthesis and secretion of fibronectin and addition of gremlin to this regime increased synthesis and secretion of fibronectin.

Conclusions: Elevated TGF-ß2 in the glaucomatous ONH may induce the secretion of the BMP antagonist gremlin that blocks the inhibitory action of BMP-4 leading to increased ECM deposition. ECM modulation by gremlin provides a novel therapeutic target for glaucoma.

Sponsor: *Glaucoma Research, Alcon Research Ltd., Fort Worth, TX.*

711 *(Poster)*

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GENDER-RELATED EXPRESSION AND COMPARITIVE SUBCELLULAR LOCALIZATION OF ESTROGEN RECEPTOR BETA ISOFORMS IN CULTURED NORMAL HUMAN LENS EPITHLIAL CELLS

Purpose: Purpose: Wild type estrogen receptor beta (wtER-ß1) and its splice variants (ER-ß2-5) coexist in human lens, as well as in cultured SV-40 transformed human lens epithelial cells (HLE-B3) (Exp Eye Res. 81:165-175; 2005). 17-beta estradiol (E2) modulates the degree of oxidative stress-induced depolarization of mitochondrial membrane potential (MMP) in HLE-B3 cells, following H2O2 insult, by activation of mitogen-activated protein kinase (MAPK) (Mitochondrion 5:235-247; 2005). This study resolved whether gender played a role in the protection mechanism based upon differences in ER-ß isoform expression, receptor localization in mitochondria and response to estrogen-mediated mitochondrial protection against oxidative stress employing cultured populations of normal male and female human lens epithelial (nHLE) cells.

Methods: Methods: : nHLE cell cultures were prepared from explants of post-mortum male and female donors across a wide age distribution. A triple primer PCR assay (Exp Eye Res. 81:165-175; 2005) was used to determine the proportional distribution of the receptor isoforms (wtER-ß1, ß2 and ß5) from the total ER-ß message pool in male and female cell cultures. Subcellular localization of ERß isoforms was determined using conventional immunofluorescence techniques and affinity purified polyclonal antibodies specific for wtER-ß1, ß2 and ß5. To examine changes in MMP, the potentiometric fluorescent compound, JC-1, was used after cell cultures were exposed to peroxide ± pretreatment with E2.

Results: Results: Male and female nHLE cells express wtER-ß1, ER-ß2 and ER-ß5 splice variants in similar ratios. Confocal microscopy and immunofluorescence revealed localization of the wild-type receptor in both the peripheral mitochondrial arrays and perinuclear mitochondria (along with weaker internal nuclear staining) of both male and female nHLE cells. The ER-ß2 and ER-ß5 isoforms were distributed in the cytosol; no association with the mitochondria was detected. Both male and female nHLE cells treated with E2 (1µM) showed similar levels of protection against oxidative stress.

Conclusions: Conclusions: While we have yet to establish whether wtER-ß1 (in mitochondria) plays a definitive role in the E2-mediated mitochondrial protection mechanism, these observations establish that prevention of depolarization of the MMP must be regarded as gender-independent.

Sponsor: *NIH*

714 *(Poster)*

Author: domalapalli kumar
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D. Maneesh Kumar, Neeraj Agarwal

NEUROPROTECTIVE MECHANISMS OF THE NON-FEMINIZING ESTROGEN ANALOGUE ZYC-3 AGAINST GLUTAMATE-INDUCED CYTOTOXICITY OF RGC-5 CELLS

Purpose: Glaucoma is a family of eye disorders whose ultimate cause of vision loss is apoptosis of retinal ganglion cells, believed to be induced by overwhelming oxidative stress. Although the presence and role of elevated intravitreal glutamate levels remains debated, subacute challenge of RGC-5 rat retinal ganglion cells with L-glutamic acid remains a reproducible method of high throughput study of oxidative challenge. From this perspecive, the work presented here was designed to examine the efficacy of ZYC-3, a synthetic estrogen analogue, as a neuroprotectant in an in vitro model of glaucoma.

Methods: RGC-5 cells were treated with L-glutamic acid (5 mM). The mechanisms of glutamate-induced oxidative cytotoxic damage and neuroprotection with ZYC-3 were assessed by measuring glutamate/cysteine anti-porter levels and activity, g-glutamylcysteine synthetase levels, and glutathione levels. In addition the levels and activity of glutathione peroxidase and glutathione reductase were determined. The mitochondrial membrane potential, against glutamate challenge, was determined using live cell confocal microscopy with JC-1 mitochondrial dye.

Results: Glutamate challenge to RGC-5 cells resulted in decreased g-glutamylcysteinsynthetase levels, decreased 35S-cysteine uptake, lowering of glutathione levels, and in a loss of mitochondrial membrane potential. The inclusion of ZYC-3 reversed the effects of glutamate cytotoxicity by the mechanisms examined in glutamate challenge.

Conclusions: Glutamate cytotoxicity resulted in oxidative damage of RGC-5 cells via loss of mitochondrial membrane potential, and the glutathione synthesis and utilization pathways. Furthermore, the data support the hypothesis that ZYC-3 may be useful in the neuroprotection of retinal ganglion cells in ocular pathologies such as glaucoma.

Sponsor: *N/A*

715 *(Oral)*

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Department: Biomedical Sciences
Classification: GSBS Student
Jwalitha Shankardas, Michellle Senchyna** and S.Dan Dimitrjevich* *Graduate School of Biomedical Sciences, Fort Worth, TX **Alcon Laboratories,Fort Wort, TX*

SUPPRESSED 14-3-3 SIGMA EXPRESSION EXTENDS THE IN VITRO LIFESPAN OF HUMAN CORNEAL AND CONJUCTIVAL EPITHELIAL CELLS.

Purpose: To demonstrate that the suppression of 14-3-3 sigma expression leads to the extended in vitro life span corneal and conjunctival epithelial cells, and "engineer" cell lines with high proliferative potential and capable of differentiation. To study the FTTS expression in postnatal FTTS mutant heterozygote (Er+) mouse.

Methods: Primary corneal and conjunctival epithelial cells, cultured in serum free defined medium, were tranfected with si-RNA (01) and FTT sigma antisense (SAS) using Amaxa nucleoporation technology. Extension of life span was determined by tracking cell numbers during serial passaging. Expression of FTTS, cell cycle proteins (P53 Rb, P63), cytokeratins and involucrin were determined by western blot analysis of cell lysates and indirect immunofluoroscense of cultured cells. Response to DNA damage (treatment with adriamycin - 0.2 ?grams/ml) was followed over 72 hours. Apoptotic cells were detected using nuclear stating with Propidium Iodide. Deparaffinized tissue sections obtained from 3 days old Er+ mice and age matched normal controls were screened for the FTT expression.

Results: "Knockdown" of FTTS expression by RNAi and antisense strategy resulted in extension of in vitro lifespan of corneal and conjuctival (?) epithelial cells. These cells express the epithelial cell markers that are typical of wild type cells. Stable, constitutive suppression of FTTS does not inactivate P53, Rb and P63 oncogenes but produced a stable cell line (over 60 population doublings). Although these cell line cells do not repair DNA damage via the P53 pathway and enter apoptosis, in the presence of serum and high calcium levels they express of involucrin and cytokeratin AE5. The 3-day old Er+ mouse corneas show two layers of epithelial cells.

Conclusions: We have shown that in corneal and conjunctival epithelial cells suppression of FTTS trnaslation extends proliferative in vitro life span. When exposed to appropriate conditions FTTS cell line expresses increasing levels of differentiation markers. We have began studying the mechanism of FTTS role in cell differentiation. Since the eyes in 3 day old mice are still closed we expect an immature corneal epithelium (2 layers) and no expression of FTTS in both Er+ and control animals.

Sponsor: *N/A*

712 *(Oral)*

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SIGNALING MECHANISMS OF MITOCHONDRIA ASSOCIATED RAT RETINAL GANGLION CELL DEATH IN OPTIC NEUROPATHIES

Purpose: Optic neuropathies are a group of ocular diseases that progress as a gradual neurodegeneration culminating in vision loss due to the eventual death of retinal ganglion cells (RGCs.) There is evidence implicating oxidative stress in many neurodegenerative diseases. We hypothesize that mitochondrial distress results in retinal ganglion cell death in optic neuropathies and that Iodoacetic acid (IAA) inhibits ATP generation resulting in mitochondrial dysfunction and generation of ROS.

Methods: Transformed rat retinal ganglion cells (RGC-5) were treated with varying concentrations of IAA from 2 to 10 μM for a period of 24 hours. Antioxidants N-Acetyl Cysteine (NAC) (2mM) and Thiourea (10mM) were administered along with IAA. Cell viability was assessed by the neutral red dye uptake assay and/or by Calcein AM assay. Morphological changes were assessed by microscopy. Changes in Mitochondrial Membrane Potential (MMP) were determined by JC-1 fluorescent dye with an argon laser on a Zeiss LSM confocal microscope. Erk phosphorylation was determined by western blot analysis using Phospho-p44 and p42 antibody. Involvement of reactive oxygen species (ROS) was determined by H2DCF (dichlorofluorescein)-DA assay, and caspase-3 by fluorescent assay.

Results: There was a dose dependent loss of cell viability by IAA treatment (2-10 μM) of RGC-5 cells. At a 6μM concentration in solution, IAA reduced cell viability by 50%. The antioxidants N-Acetyl Cysteine (2mM) and Thiourea (10mM) rescued RGC-5 cells from IAA induced cell death. IAA induces caspase activation in a dose dependent manner. IAA does not induce the activation of caspases in the presence of exogenous antioxidants NAC and Thiourea. IAA reduced the mitochondrial membrane potential in RGC-5 cells, which was rescued by NAC. NAC induces the phosphorylation of ERK 1 and 2.

Conclusions: IAA is cytotoxic to retinal ganglion cells. IAA does not induce death in retinal ganglion cells in the presence of exogenous antioxidants, N-acetyl cysteine and Thiourea. IAA induced RGC cell death involves oxidative stress that can be reversed by antioxidants. IAA creates a loss of MMP while NAC maintains the MMP. IAA does not result in phosphorylation of ERK1 and 2. NAC induces the phosphorylation of ERK1 and 2 in a time dependent manner while thiourea does not. NAC upregulates the expression of Bcl-2 in RGC-5 cells. Taken together, these results suggest IAA induced mitochondrial cytotoxicity involves Erk pathway.

Sponsor: *N/A*

713 *(Poster)*

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DIFFERENTIAL EXPRESSION OF PROPROTEIN CONVERTASE PACE4 IN THE RETINA AND OPTIC NERVE HEAD

Purpose: The proprotein convertases (PC) are a family of subtilisin/kexin-like serine proteases that are known to process a wide variety of proteins into active forms. Recent studies have suggested that PC modulation may be involved in many conditions including tumor invasiveness and nervous system damage. PACE4 regulation has been demonstrated to modulate the bioavailability of transforming growth factor (TGF) related proteins. The purpose of this study is to determine the expression patterns for PACE4 in the human retina and optic nerve head.

Methods: RNA was isolated from postmortem human brain, retina, and optic nerve head, and mRNA expression levels for members of the PC family were determined using Quantitative real-time polymerase chain reaction (QRT-PCR). Protein lysates generated from postmortem human retina and optic nerve were analyzed for PACE4 expression via immunoblotting. Immunohistochemistry was used to localize the PC family in the retina and optic nerve head.

Results: Immunohistochemistry for PC1 demonstrates strong staining throughout the retina, with decreased expression in GFAP positive cells in the ganglion cell layer/nerve fiber layer. PC1 mRNA expression is highest in postmortem brain, and 1.5 fold lower in ONH relative to retina. PC2 expression is highest in retina, and is approximately 4.5 fold lower in ONH relative to retina. Furin and PC7 mRNA levels are not significantly different between retina and ONH, but both demonstrate higher expression compared to postmortem brain. PACE4 mRNA expression is 1.7 and 2.5 fold higher in postmortem human ONH relative to postmortem retina and brain, respectively. Immunoblotting for PACE4 demonstrates prominent bands at approx 70 and 44 kDa in retina and optic nerve lysates.

Conclusions: This study suggests that PACE4 may be an important regulator in the processing and maturation of growth factors in optic nerve head glia, and may be an upstream mediator of gliosis and extracellular matrix remodeling of the ONH.

Sponsor: *Neurobiology of Aging training grant*

710 *(Oral)*

Author: Gulab Zode
Presenter: Gulab Zode
Department: Cell Biology and Genetics
Classification: GSBS Student
Gulab Zode, Abbot F. Clark, and Robert J. Wordinger Department of Cell Biology and Genetics, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX., Glaucoma Research, Alcon Research Ltd., Fort Worth, TX.

BONE MORPHOGENETIC PROTEIN 4 INHIBITS TGF-B2 SIGNALING IN OPTIC NERVE HEAD ASTROCYTES AND LAMINA CRIBROSA CELLS: EXTRACELLULAR MATRIX MODULATION BY GREMLIN IN GLAUCOMA

Purpose: The characteristic cupping of the optic nerve head (ONH) in glaucoma is associated with elevated TGF-ß2 expression and altered synthesis and deposition of extracellular matrix (ECM) proteins. Bone morphogenetic proteins (BMP) have been reported to selectively inhibit the fibrotic action of TGF-ß2. We hypothesize that in glaucoma, elevated TGF-ß2 levels in the ONH induce expression of gremlin, a secreted BMP antagonist, which selectively blocks BMP antagonism of TGF-ß2 signaling. This antagonism would result in increased ECM synthesis and deposition. The purpose of this initial study was to demonstrate that A) TGF-ß2, BMP-4, fibronectin, and gremlin is localized in human ONH, B) TGF-ß2 treatment upregulates gremlin expression, and C) gremlin inhibition of BMP-4 increases TGF-ß2- stimulated fibronectin (FN) production in cultured human ONH astrocytes and LC cells.

Methods: Immunohistochemistry was used to examine TGF-ß2, BMP-4, fibronectin, and gremlin protein level in age matched normal and glaucomatous ONH tissues (N=3). Human ONH astrocytes (N=4) and LC cells (N=5) were utilized. Effect of TGF-ß2 (5ng/ml) on gremlin expression was examined by QPCR and western blot analysis. To examine BMP-4 inhibition of TGF-ß2 signaling, ONH astrocytes and LC cells were treated exogenously with a) TGF-ß2 (5ng/ml) or BMP-4 (10ng/ml), b) TGF-ß2 and BMP-4, c) TGF-ß2, BMP-4 and gremlin (300ng/ml), and fibronectin content was assessed by western blot.

Results: Immunohistochemistry analysis demonstrated that protein for TGF-ß2, fibronectin, and gremlin is increased in glaucomatous ONH tissues. BMP-4 is localized in ONH and does not seem to be increased in glaucomatous ONH. Exogenous TGF-ß2 treatment increases gremlin mRNA and protein secretion in conditioned media of ONH astrocytes and LC cells. Western blot analysis demonstrated that TGF-ß2 increases fibronectin secretion significantly and that exogenous BMP-4 does not have significant effect on fibronectin synthesis and secretion. Coincubation of BMP-4 and TGF-ß2 reduced TGF-ß2 stimulated synthesis and secretion of fibronectin and addition of gremlin to this regime increased synthesis and secretion of fibronectin.

Conclusions: Elevated TGF-ß2 in the glaucomatous ONH may induce the secretion of the BMP antagonist gremlin that blocks the inhibitory action of BMP-4 leading to increased ECM deposition. ECM modulation by gremlin provides a novel therapeutic target for glaucoma.

Sponsor: *Glaucoma Research, Alcon Research Ltd., Fort Worth, TX.*

711 *(Poster)*

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Presenter: James Flynn
Department: Cell Biology and Genetics
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J.M. Flynn 1A, S.Dimitrijevich 1B, M.Younes 2, G.Skliris 3, L.C. Murphy 3, P.R. Cammarata 1A. A Cell Biology/Genetics, B Intergrative Physiology, 1 Uni. of North Texas HSC, Fort Worth, TX; 2 Pathology, Baylor College of Medicine, Houston, TX; 3 Biochemistry and Molecular Biology, Uni. of Manitoba, Winnipeg, MB, Canada.

GENDER-RELATED EXPRESSION AND COMPARITIVE SUBCELLULAR LOCALIZATION OF ESTROGEN RECEPTOR BETA ISOFORMS IN CULTURED NORMAL HUMAN LENS EPITHLIAL CELLS

Purpose: Purpose: Wild type estrogen receptor beta (wtER-ß1) and its splice variants (ER-ß2-5) coexist in human lens, as well as in cultured SV-40 transformed human lens epithelial cells (HLE-B3) (Exp Eye Res. 81:165-175; 2005). 17-beta estradiol (E2) modulates the degree of oxidative stress-induced depolarization of mitochondrial membrane potential (MMP) in HLE-B3 cells, following H2O2 insult, by activation of mitogen-activated protein kinase (MAPK) (Mitochondrion 5:235-247; 2005). This study resolved whether gender played a role in the protection mechanism based upon differences in ER-ß isoform expression, receptor localization in mitochondria and response to estrogen-mediated mitochondrial protection against oxidative stress employing cultured populations of normal male and female human lens epithelial (nHLE) cells.

Methods: Methods: : nHLE cell cultures were prepared from explants of post-mortum male and female donors across a wide age distribution. A triple primer PCR assay (Exp Eye Res. 81:165-175; 2005) was used to determine the proportional distribution of the receptor isoforms (wtER-ß1, ß2 and ß5) from the total ER-ß message pool in male and female cell cultures. Subcellular localization of ERß isoforms was determined using conventional immunofluorescence techniques and affinity purified polyclonal antibodies specific for wtER-ß1, ß2 and ß5. To examine changes in MMP, the potentiometric fluorescent compound, JC-1, was used after cell cultures were exposed to peroxide ± pretreatment with E2.

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Sponsor: *NIH*

712 *(Oral)*

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Sponsor: *N/A*

713 *(Poster)*

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Sponsor: *Neurobiology of Aging training grant*

714 *(Poster)*

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Sponsor: *N/A*

715 *(Oral)*

Author: Jwalitha Shankardas
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Department: Biomedical Sciences
Classification: GSBS Student
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Sponsor: *N/A*

716 *(Poster)*

Author: Zhaohui Wang

Presenter: Zhaohui Wang

Department: Cell Biology and Genetics

Classification: GSBS Student

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MAPK PATHWAYS MODULATE RPE CELL RESPONSE TO OXIDATIVE STRESS

Purpose: Age-related Macular Degeneration (AMD), a major cause of irreversible visual loss in the elderly, is characterized by early deposits of drusen, progressive dysfunction of the retinal pigment epithelium (RPE), and eventual loss of RPE and photoreceptor cells. Although multi-factorial in nature, oxidative stress (OS) is a major contributing factor in its etiology. In this study, we investigated the effects of OS on RPE cells and the role of MAPKs in the development of AMD.

Methods: A well-characterized RPE cell line (ARPE-19) was exposed to a glucose-based oxidant-generating system catalyzed by glucose oxidase (GO/G). ARPE-19 cells were characterized for morphological changes, mitochondrial membrane permeability (MMP), and cell survival following transient (non-lethal OS) or continuous exposure (lethal OS) to GO/G. The effects of OS on MAPK activity were determined by assaying for p38 or p42/p44 MAPK phosphorylation and the use of pharmacological inhibitors and expression plasmids.

Results: ARPE-19 cells exposed to non-lethal OS promoted actin-filament reorganization, membrane blebbing, and exosome formation--in the absence of degenerative changes. With continuous OS exposure, progressive membrane/cytoskeletal changes were accompanied by increased MMP and apoptosis. Exposure to OS promoted increased phosphorylation of p38 MAPK and hsp27, a cytoprotective molecular chaperone and downstream target of p38 MAPK involved in cytoskeletal organization. On the other hand, p42/p44 MAPK exhibited biphasic response to OS exposure—an early increase in phosphorylation followed by a decrease below control levels in dying cells. Pharmacological inhibition of p38 MAPK or p42/p44 MAPK activation by MEK1 overexpression suppressed the morphological and biochemical changes and cell death.

Conclusions: In conclusion, non-lethal OS activates early cytoprotective responses involving morphological and biochemical changes including activation of p38 MAPK/hsp27 and p42/p44 MAPK pathways. These prosurvival responses, however, paradoxically promote extracellular release of cellular fragments and secretory protein components of drusen. Inactivation of p42/p44 MAPK, but not of p38 MAPK, appears to be critically responsible in OS-induced cell death. While further investigations are needed to establish their exact roles in RPE response to OS, our study suggests that p38 MAPK and p42/p44 MAPK pathways could serve as potential targets for pharmacological intervention of AMD.

Sponsor: *N/A*

800 *(Poster)*

Author: John Licciardone

Presenter: John Licciardone

Department: Osteopathic Research Center

Classification: Faculty

John C. Licciardone, D.O., M.S., M.B.A., Osteopathic Research Center, University of North Texas Health Science Center, Fort Worth, TX 76107; Roberto Cardarelli, D.O., M.P.H., Department of Family and Community Medicine, University of North Texas Health Science Center, Fort Worth, TX 76107; Carol Knisley, Osteopathic Research Center, University of North Texas Health Science Center, Fort Worth, TX 76107.

OSTEOPATHIC MEDICINE AND PRIMARY CARE

Purpose: Osteopathic Medicine and Primary Care is an open access, peer-reviewed, online journal that aims to encompass all aspects of family medicine, internal medicine, pediatrics, and obstetrics and gynecology. The journal will also cover health services and public health research that addresses primary care issues. In addition to the aforementioned themes, the journal will also cover uniquely osteopathic topics, such as osteopathic manipulative treatment and its mechanisms of action, reliability of palpatory findings, clinical outcomes, and efficacy in treating a variety of musculoskeletal conditions or related disorders.

Methods: Osteopathic Medicine and Primary Care will consider for publication the following types of articles. Research: reports of data from original research. Reviews: comprehensive, authoritative descriptions of any subject within the journal's scope. Reviews can cover any topical themes such as basic science and clinical reviews, ethics, pro/con debates, equipment reviews, and thematic series to highlight specific topics in the field. Commentaries: short, focused, and opinionated articles on any subject within the journal's scope. Short reports: brief reports of data from original research, usually about 1500 words. Study protocols: proposed or ongoing research, providing a detailed account of the hypothesis, rationale, and methodology of the study.

Results: Osteopathic Medicine and Primary Care was launched in January 2007. Seven articles were published as of February 15, 2007. These and subsequent articles will be indexed in PubMed and archived in PubMed Central.

Conclusions: Investigators and scholars in the fields of osteopathic medicine and primary care have a publishing option that provides thorough and fair peer review, expedited publication, open access and high visibility, and retention of copyrights to authors.

Sponsor: *Osteopathic Heritage Foundations*

801 *(Poster)*

Author: Mayra Rodriguez

Presenter: Mayra Rodriguez

Department: Research

Classification: GSBS Student

Mayra Rodriguez, BS Roberto Cardarelli, DQ, MPH, FAAFP Ana Luz Chiapa, MS University of North Texas Health Science Center at Fort Worth, Department of Family & Community Medicine, Division of Education and Research, Fort Worth, Texas 76107

THE RELATIONSHIP BETWEEN CANNABIS USE, DIETING AND EXERCISE

Purpose: The active chemical, delta-9-tetrahydrocannabinol binds to receptors in the cerebellum and basal ganglia producing a euphoric effect. Although short lasting, it reduces anxiety while increasing self-esteem. Isolation, rejection, anxiety, and depression lead teenagers to become psychologically dependent. However, few studies show the relationship between dieting behaviors, physical activity and its relationship with Cannabis use. We hypothesize that an increase in dieting and strenuous physical activity increases the likely use of Cannabis among adolescents while controlling depressive behaviors.

Methods: The 2005 Youth Risk Behavior Surveillance System was used. Schools totalling 159 participated, gathering 13,917 surveys. Participants were dichotomized into two age groups: 12-15 and 16-19 yrs. Race/ethnitcy was coded as white, Hispanic/Latino, African-American, other. Independent variables, dichotomized yes/no included responses to fasting, taking pills, or using laxatives/vomiting to lose weight. Exercise frequency was categorized as 0 or 1-7 days in the last week. Exercising to lose weight was coded as yes or no. The dependent variable was cannabis use categorized as "never used" or "has used". Logistic regression models were developed to obtain odds ratio and 95% confidence intervals. Results were stratified by age, and complex sampling statistical techniques were used.

Results: Among adolescent users, 37.8% described their health as good while 38.0% of non users described their health as very good. However, both groups (46.9% users/46.3% non-users) were trying to lose weight($p<0.001$). Approximately 76% of users reported being physically active and 24.4% reported no exercise($p=0.77$). Among cannabis users, 38.1% felt sad or hopeless ($p=0.004$) and 24.9% had suicidal thoughts during the past year ($p<.001$). Age was shown to modify ($p=0.01$) the association between the primary independent (fasting to lose weight) and dependent variable (cannabis use). Univariate regression showed that among younger adolescents those trying to lose weight were 1.33 times more likely to use cannabis compared to those not trying to change body weight. The group who took laxatives or reported self-induced vomiting (OR=4.41), used pills(OR=5.12) were more likely to use cannabis.

Conclusions: Results suggest younger adolescents are more likely to engage in unhealthy behaviors such as dieting and cannabis use. Older adolescents are less influenced by others having gained self-discipline.

Sponsor: *N/A*

802 *(Poster)*

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HLA-C ALLELES 0602 AND 0701 HAVE OPPOSING EFFECTS ON SEVERITY OF PSORIASIS DURING PREGNANCY

Purpose: Psoriasis is a chronic, relapsing and remitting skin and joint disease with high morbidity and unknown etiology influenced by heritable, immunologic, hormonal, and environmental factors. Presentation is diverse representing distinct subtypes. Psoriasis has been shown to improve in about one-third of patients during pregnancy. Improvement has been previously associated with the HLA-Cw*0602 allele. As the etiology and pathophysiology of psoriasis are established, targeted treatments will be developed to improve morbidity and provide an eventual cure.

Methods: 138 patients with onset of psoriasis prior to pregnancy in the Utah Psoriasis Initiative (UPI) completed written or phone survey, physical assessment, and HLA-C genotyping. 37, 22, and 41 percent reported improvement, exacerbation, or no change of psoriasis severity during pregnancy, respectively.

Results: HLA-Cw*0602 was more common in patients reporting improvement versus those who worsened (n=80, chi2=6.52, p=0.01). HLA-Cw*0701 was more common in patients who reported worsening during pregnancy (n=80, chi2=3.99, p=.05). Exacerbation of psoriasis was also associated with postpartum state (n=56, chi2=2.95, p=.003), menopause (n=58, chi2=29.49, p<.001), and bilateral oophorectomy (n=20, chi2=7.20, p=.007). The analysis was repeated when patients were grouped into HLA-C functional groups C1 (including HLA-Cw*0701, *0101, *0302, *0303, *0304, *0701/06/18, *0702, *0704/11, *0801, *0802, *1202, *1203, *1402, and *1601), and C2 (including Cw*0602, *0611, *1502, *1505, *1602, and *1701/02/03). The majority of patients who exacerbated during pregnancy were found to be homozygous for C1, while patients who experienced remission were more frequently heterozygous or homozygous for C2 (chi2=5.76, p=.02).

Conclusions: HLA-C is expressed on placental tissue and thought to play a central role in natural killer (NK) cell fetal allograft tolerance through interaction with Killer Immunoglobulin-Like Receptors (KIRs). C1 interacts with KIR2DL2, KIR2DS2, and KIR2DL3 distinct from KIR2DL1 and KIR2DS1 which interact with C2 alleles. We conjecture that the HLA-Cw*0602 allele may preferentially inhibit NK cells during pregnancy and could play a role in improvement of psoriasis, with the reverse being true for HLA-Cw*0701.

Sponsor: *N/A*

803 *(Poster)*

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A NEW SILENT EPIDEMIC: ARE PRIMARY CARE CLINICIANS DOING ENOUGH TO DETECT THE EARLY ONSET OF CHRONIC KIDNEY DISEASE IN HIGH RISK PATIENTS?

Purpose: In order to help standardize clinical practice procedures for the early detection and screening of Chronic Kidney Disease (CKD), the National Kidney Foundation implemented the Kidney Disease Outcomes Quality Initiative (KDOQI) Guidelines. The purpose of this study was to evaluate the clinical awareness and knowledge that primary care clinicians have of recognizing and screening high risk individuals as outlined by the KDOQI guidelines.

Methods: A 10-question online survey consisting of Likert scale and multiple choice questions was utilized to gather information from the respondents regarding their opinion of the level of importance of screening and their familiarity and knowledge of the guidelines. The researcher developed the survey based upon the screening recommendations outlined by the KDOQI guidelines. The survey was sent via email to approximately 300 members of the American Academy of Family Physicians and 650 members of the American Academy of Physician Assistants. After data collection was completed, SPSS 14.0 software was used to perform statistical analysis using Chi-Squared, t-test and ANOVA.

Results: A total of 246 respondents participated in the survey. A majority of respondents (87.8%) agreed that early screening and detection was as important as screening for other more prevalent diseases. However, most respondents (77.3%) did not consider themselves familiar with the KDOQI Guidelines. This self-described lack of familiarity of the guidelines became evident when looking at the participant's average scores within the knowledge portion of the survey. Overall, respondents scored an average of 4.98 (of 11 points) on the total knowledge score. Most respondents were able to correctly identify the recommended risk factors for CKD but had trouble distinguishing these from the choices which were not recommended factors (p<0.0001, p>0.05 respectively). The majority of the respondents were unable to correctly select the correct lab measures for initial screening and evaluation of persistent proteinuria (p<0.001, p<0.001 respectively) and were unaware of which level of CKD to refer to a nephrologists (p<0.001).

Conclusions: The study found that a majority of the respondents did not have sufficient knowledge about the KDOQI guidelines' recommendations. This suggests that more educational measures need to be implemented to increase clinician awareness. Future recommendations include analysis of educational training outcomes and evaluation of clinic screening procedures.

Sponsor: *N/A*

900 *(Poster)*

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CLONING AND CHARACTERIZATION OF THE LIGAND FOR NATURAL CYTOTOXICITY RECEPTOR, NKP44

Purpose: The over all purpose of this study is to understand the molecular mechanism by which Natural Killer cells recognize and kill target cells. Natural killer (NK) cells represent a specialized lymphoid population that mediate innate immune responses against tumor or virally infected cells. NK cell cytotoxicity is closely regulated by numerous inhibitory and activating receptors. The inhibitory receptors, KIRs (killer cell Ig-like receptors) recognize Major Histocompatibility Complex (MHC) I molecules. The activating receptors are the Natural Cytotoxicity Receptors (NCRs), 2B4, and NKG2D. The NCRs play a key role in recognition and killing of MHC I deficient cells and include the receptors NKp30, NKp46, and NKp44. The ligands for the NCRs are not yet known. Among the NCRs, NKp44 is of particular interest because it is only expressed on activated NK cells, and is implicated in increased cytotoxicity and HIV infection. In this study we intend to identify and clone the ligand for NKp44.

Methods: NKp44-IgG fusion protein was produced by transiently transfecting B16 cells with DNA encoding the extra cellular domain of NKp44 and the FC portion of IgG. This fusion protein was utilized to screen cell lines for expression of the NKp44 ligand. Messenger RNA was isolated from DB cells and constructed into a directional complimentary DNA (cDNA) library for transformation into ultra competent E. coli bacteria. The cDNA library was amplified and then transiently transfected into B16 cells. The cDNA encoding the NKp44 ligand was isolated utilizing mammalian expression cloning.

Results: We have generated a soluble NKp44-IgG fusion protein. Using this recombinant fusion protein we identified a cell line (DB) that express the ligand for NKp44 by FACS analysis. We also constructed a cDNA library using mRNA from DB cells.

Conclusions: Characterization of this ligand and its interaction with NKp44 will help to develop new strategies in immunotherapy of cancer using NK cells.

Sponsor: *NIH*

901 *(Poster)*

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CYTOKINE DERIVED DIFFERENTIATED DENDRITIC CELLS HAVE ALTERED RESPONSES TO MYCOPLASMA PULMONIS

Purpose: Mycoplasma pulmonis causes respiratory disease in mice with similarities to human Mycoplasma pneumoniae infections. The adaptive immune response against mycoplasma in the lungs can be either protective or damaging. Dendritic cells (DCs), potent antigen presenting cells, may influence the adaptive immune response to mycoplasma in the lungs. To understand these immune responses, we proposed to examine the influence mycoplasma has on differentiated DC populations. We hypothesized that DCs cultivated in different cytokine environments have opposing responses to mycoplasma.

Methods: Bone marrow cells were isolated from the tibiae and femora of 6-8 wk old Balb/c mice. The cells were grown in the presence of differing cytokines [GM-CSF only, GM-CSF and IL-4, and GM-CSF, TGF-ß1 and IL-10] for 6 d to produce three classes of DCs designated as GMDC, G4DC, and T10DC. On day 6, the DCs were sorted using the CD11c+ magnetic beads and the AutoMACs. All CD11c+ cells were stimulated for 24 hrs with LPS, FSL-1, whole mycoplasma and culture medium only to determine cytokine responses. The IL-12, IL-10 and TNF-a cytokine expression was determined using ELISA from the DC supernatants.

Results: The GMDC group showed the highest production of IL-12 and TNF-a and a median level of IL-10. The T10DC group showed a median level of IL-12 and TNF-a production, but the highest level of IL-10 production. Although the unstimulated G4DC group showed the highest levels of co-stimulatory molecules as indicated by flow cytometry, the G4DC showed the lowest levels of IL-12, TNF-a, and IL-10 responses. Cytokine mRNA expression, as determined by quantitative real-time PCR roughly corresponded with protein production.

Conclusions: These data indicate differentiated classes of DCs developed by prior exposure of cytokines react differently to mycoplasma. These differences in mycoplasma response may affect the generation of adaptive immunity, which may provide insights for a novel vaccine approaches that optimize protective immunity while minimizing immunopathology. Future studies consist of examining the influences each DC group has on T-cell responses to mycoplasma.

Sponsor: *NIH 1RO1 HL069431*

902 *(Poster)*

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FUNCTION AND SIGNALING OF THE LLT1 RECEPTOR ON NATURAL KILLER CELLS

Purpose: NK cells are cells of the innate immune system that form the first line of defense against cancer and viral infections. NK cell functions are regulated by a balance between activating and inhibitory signals received through surface receptors. We have previously identified a lectin-like transcript 1 [LLT1] from a human NK cell cDNA library. LLT1 is expressed on NK cells, monocytes, B cells and T cells. Furthermore, Mathew et al. have shown that LLT1 ligation on NK cells is a potent stimulator of IFN-gamma secretion. LLT1 ligation has no effect upon the cytotoxic properties of NK cells. The natural ligand of LLT1 has recently been identified as NKRP1A, an important regulatory receptor on NK and T cells. A recent report shows that LLT1 is expressed on osteoclasts and it inhibits their formation and function. We hypothesize that LLT1 exhibits diverse functional roles on various cell types, and may employ multiple intracellular signaling strategies to accomplish this. Functioning as an immune modulator, LLT1 may possibly link a regulatory feedback loop between activated NK cells and macrophages.

Methods: A mouse monoclonal antibody specific for human LLT1 was generated using soluble LLT1 and LLT1 peptides. Hybridomas were screened via ELISA and flow cytometric analysis of PBMCs. For functional assays, a soluble NKRP1A fusion protein was also generated. Numerous cell lines were stained with this fusion protein and antibody to analyze the expression and function of LLT1 on various cell types. Additionally, a promonocytic cell line U937 was assayed for LLT1 expression by flow cytometry under multiple permutations, including incubation with IFN-gamma and anti-LLT1 IgG.

Results: LLT1 dependent NK cell IFN-gamma secretion is dependent upon Src-family Protein Tyrosine Kinase activity and additional downstream signaling mechanisms. 100% of unstimulated U937 express LLT1. Activation of U937 cells by IFN-gamma induces a five-fold increase in LLT1 expression.

Conclusions: The stimulation of IFN-gamma secretion by LLT1 on NK cells and its wide expression on cells of the adaptive immune response indicate that LLT1 may function as a link between innate and adaptive immune responses.

Sponsor: *NIH grant CA85753; NSF Project SCORE*

903 *(Poster)*

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FUNCTIONAL ROLE OF HUMAN 2B4 (CD244) ISOFORMS IN NATURAL KILLER CELLS

Purpose: Natural killer (NK) cells are a subpopulation of lymphocytes that play an important role against tumor metastasis and various viral and bacterial infections. NK cell functions are controlled by a balance between positive and negative signals through various receptors. We have identified, cloned and characterized the 2B4 (CD244) receptor in mice and human. 2B4 is involved in killing cancer cells and virus-infected cells by NK cells. 2B4 is a counter-receptor for CD48 and recent findings show that 2B4-CD48 interactions play an important role in NK, T and B cell functions. In humans, two isoforms of 2B4, h2B4-A and h2B4-B, are expressed that differ in the extracellular domain. The difference between the h2B4-A and h2B4-B is due to differential splicing of exon 3 of the human 2B4 coding region. h2B4-B contains the first 15 nucleotides of exon 3 that result in the addition of five amino acid residues in the C2 region of the extracellular domain. The purpose of the investigation was to study the functions of h2B4-A and h2B4-B.

Methods: Using sequence specific primers, the extracellular domain of h2B4-A and h2B4-B was amplified and cloned into a pCD5Ineg1 vector. Transient transfection of pCD5Ineg1-h2B4-A-Fc and pCD5Ineg1-h2B4-B-Fc was performed using B16 cells to produce secreted recombinant fusion proteins of h2B4-A and h2B4-B. The recombinant fusion proteins were then used for subsequent analysis by flow cytometry and human IFN-gamma ELISA.

Results: We found that soluble h2B4-A-Fc fusion protein containing the extracellular domain of h2B4-A bound to NK92-CD48 with greater affinity than the h2B4-B-Fc fusion protein. It was also shown that h2B4-A-Fc fusion protein interaction with CD48 on NK92 resulted in a greater IFN-gamma production than did h2B4-B-Fc.

Conclusions: Our data demonstrate that these two isoforms differ in their binding affinity for CD48 and this result in differential cytokine production by NK cells. Thus differential expression of 2B4 isoforms by NK cells may regulate immune responses mediated through 2B4-CD48 interactions. This work was supported by the NIH grant CA85753.

Sponsor: *N/A*

906 *(Poster)*

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INFLUENCE OF CONTROLLABILITY OF STRESS ON PULMONARY IMMUNE RESPONSE

Purpose: The ability to control how stressful a situation is can be a determining factor in the development of asthma. We hypothesize that the ability to exert control on stress dampens cellular immune responses that are responsible in disease pathogenesis. In this study, we investigated how cellular responses were influenced in a murine model of asthma.

Methods: . Female Balb/c mice were sensitized with an experimental allergen (Ovalbumin + Alum), while being exposed to escapable and inescapable shock stress. Following sensitization, all mice were challenged intranasally with Ovalbumin. The mice able to escape shock stress demonstrated greater learned resilience and weight gain. 18 hours following OVA challenge, differential cellular staining of the bronchiolar lavage fluid was performed.

Results: The results revealed significant increases in neutrophils, macrophages and eosinophils among mice exposed to inescapable shock stress as compared to escapable and home cage controls. In the lung, flow cytometry results demonstrated a higher percentage of neutrophils among mice exposed to inescapable stress. We found no significant changes in the percentage of T cells and B cells under stress conditions.

Conclusions: Based upon these results, we found that controllability of environment determines the extent of immune responsiveness toward allergen exposure that may be associated with development of asthma.

Sponsor: *N/A*

907 *(Oral)*

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NOVEL ROLE OF NK CELLS IN THE DEVELOPMENT OF ADAPTIVE IMMUNITY AGAINST MYCOPLASMA RESPIRATOY DISEASE

Purpose: A complex regulatory balance between the detrimental and beneficial effects of immunity determines the course of mycoplasma respiratory infection. NK cells were shown to dampen innate immunity in the lungs. The purpose of this study was to determine if NK cells similarly impacted the development of adaptive immunity against mycoplasma disease.

Methods: Anti-asialo GM1 Ab was used to transiently deplete NK cells prior to nasal-pulmonary immunization with mycoplasma membrane Ag. Sham immunized mice and mice receiving only anti-asialo GM1 Ab served as controls. This treatment was repeated after 7d, and 7d later, mice were challenged with M. pulmonis. DX5+ NK cell numbers returned to normal by this day. Colony forming units (CFUs) in lungs and nasal passages were determined 3, 7 and 14 days later.

Results: There was a significant decrease (1 log) in CFUs in lungs and nasal passages of the NK cell depleted, immunized mice as compared to immunized and control mice. This effect was not seen in SCID mice indicating adaptive immunity was affected. Further support that the protection rendered, due to depletion of NK cells prior to immunization, was lymphocyte-mediated was found in studies where protection was shown to be adoptively transferred to naïve mice using total lung lymphocytes, purified T and/or Non T cells. Protection was not associated with difference in Ab titers other than IgG2a and in vitro cytokine response.

Conclusions: In conclusion, NK-like cells dampen the generation of protective adaptive immunity in the lungs and nasal passages associated with nasal-pulmonary immunization, and the effect by NK cells is on the generation of lymphoid cell mediated response. The results of these studies should provide insights into approaches to generate optimal protective immunity against mycoplasma respiratory diseases.

Sponsor: *1RO1 AI 42075*

904 *(Poster)*

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LYMPHATIC PUMP TREATMENT INCREASES T AND B LYMPHOCYTES IN THORACIC DUCT LYMPH

Purpose: Osteopathic physicians have long used lymphatic pump treatments (LPT) as treatment for edema and infection. LPT enhances lymphatic return by increasing the gradient for lymph flow and assisting the return of lymph flow from the lung, abdomen and other tissues. Although there are numerous reports of benefits of LPT, there has been little research to support this treatment.

Methods: In the current investigation, the thoracic ducts of eight anesthetized dogs were catheterized, so the effects of LPT on lymph flow and leukocyte output could be measured. Lymph flow was measured, and lymph was collected over ice under 1) baseline (resting) conditions, and 2) during application of LPT. The baseline and LPT samples were stained for viable leukocytes using Trypan Blue, and the viable cells were counted using a hemocytometer. The T cells in the samples were stained with an anti-CD3 monoclonal antibody, and B cells were stained with an anti-B cell monoclonal antibody. Flow cytometry was performed, and both the number and percentage of T and B lymphocytes was determined.

Results: LPT increased T lymphocytes from 3.55 ± 1.65 million to 9.22 ± 3.87 million cells/ml, B cells from 0.80 ± 0.36 million to 2.4 ± 0.07 million cells/ml. The numbers of B and T lymphocytes in lymph increased, but their percentage was unaltered. LPT significantly enhanced lymph flow rate from 1.13 ± 0.44 to 4.14 ± 1.29 ml/minute. Therefore, T cell and B cell flux, computed from the product of lymph flow and cell count, was significantly increased during LPT. Specifically, T lymphocyte flux increased from 7.24 ± 4.15 to 51.9 ± 26.5 cells/min during LPT, and B lymphocyte flux increased from 1.39 ± 0.75 to 11.2 ± 3.90 cells/min.

Conclusions: In conclusion, LPT significantly increased thoracic duct lymph flow, total leukocytes, and T and B lymphocytes, and leukocyte flux. (This research was supported by NIH Grant U19 AT2023-01)

Sponsor: *NIH Grant U19 AT2023-01*

905 *(Poster)*

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DIFFERENTIAL EXPRESSION OF CHEMOKINES IN PULMONARY ANTIGEN PRESENTING CELLS AFTER MYCOPLASMA PULMONIS INFECTION IN MICE

Purpose: Mycoplasma pulmonis causes chronic lung disease in mice. Studies in our laboratory demonstrate that antigen presenting cells (APC) can mediate pulmonary immune responses against M. pulmonis infection. We found that dendritic cells (DC) and macrophages (MC) were the major pulmonary APC after M. pulmonis infection. The current focus of our study is to determine the role of chemokine/cytokines from APC involved in recruitment/activation of other immune cells.

Methods: Gene microarray and real-time PCR were performed to examine mRNA expression of chemoikine genes in DC and MC isolated from infected lung after 14 days of infection. To confirm these expressions in translation level, protein levels were analyzed using ELISA (enzyme immunosorbent assay). Flow cytometry analysis was used to analyze expressions of chemokine receptor on T cells.

Results: Different expression pattern of chemokines genes was detected in DC and MC using gene array data. Based on this results primers for real time RT-PCR were chosen. Real time RT-PCR showed expression of Fc?R1-?, MIF and CXCL10 were significantly increased in both DC and MC whereas mRNA expression of CCR1 and CCL4 were significantly increased only DC after 14 days of infection. ELISA showed that CXCL10 was only secreted co-culture with APC and T cells from infected mice lung. Moreover, co-culture of infected DC and T cell showed highest level of CXCL10 secretion. CCL4 was produced in non APC and T cell culture. However, CCL4 level was increased in infected APC and T cell co-culture. Flow cytometry data showed that CXCR3 expression was increased by 1% in lung CD4/CD8 T cell after 7 day of infection whereas didn't show any difference after 14 day infection. CCR5 expression didn't showed differences in both 7 days and 14 days after infection.

Conclusions: These data suggest that each APC has different pattern of chemokine/cytokine secretion and receptor expression after M. pulmonis infection and receptor expression is also important factor to determine interaction between APC and target cells. We hypothesized that these differences can trigger differential immune cell recruitment and interactions with APC during mycoplasma disease pathogenesis. Future study, we will test our hypothesis that chemokine/cytokine mRNA expression patterns reflect differences in protein production and there factors play a role in the response to M. pulmonis infection. This research is supported by NIH GRANT 1RO1HL069431-01A2.

Sponsor: *NIH 1RO1HL069431-01A2.*

908 *(Oral)*

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DIFFERENTIAL SECRETION OF IL-12 AND IL-18 BASED ON INTRACELLULAR LOCALIZATION OF LISTERIA MONOCYTOGENES

Purpose: Listeria monocytogenes (LM) is a gram-positive, intracellular bacterium that causes spontaneous abortion in pregnant women and meningitis in immunocompromised individuals. LM is commonly used to investigate the immune response to infection. Pro-inflammatory cytokines, such as IL-12 and IL-18, are secreted during LM infection to promote clearance of the bacteria through the induction of IFN-gamma. Viable LM secretes listeriolysin O (LLO) which allows the bacterium to invade the cytoplasm while neither heat-killed LM (HKLM) nor LLO deficient LM (LLO-LM) actively produces LLO. HKLM is not an effective vaccine and therefore we hypothesize that the cytoplasmic invasion of LM is necessary for the induction of a protective IFN-gamma response.

Methods: After infecting bone marrow derived macrophages (macs) and dendritic cells (DCs) with the different forms of LM, IL-12 and IL-18 were measured using ELISA. In addition, co-cultures were established to measure IFN-gamma production by splenocytes in response to infected macs and DC. For in vivo studies, C57Bl/6 mice were infected with LM, LLO-LM, or HKLM and IFN-gamma, IL-12, and IL-18 were measured.

Results: When macs or DCs are infected with LM, HKLM, or LLO-LM and co-cultured with naïve splenocytes, only macs or DCs infected with LM are able to induce IFN-gamma. ELISA data demonstrates that IL-12 is secreted from LM, HKLM, or LLO-LM infected macs and DCs, but IL-18 is only secreted from macs and DCs infected with LM. When mice are infected with HKLM or LLO-LM, there is no detectable IFN-gamma secretion and this can be attributed to a lack of both IL-12 and IL-18.

Conclusions: Therefore, in vitro IL-12 can be secreted by macs and DCs regardless of cytoplasmic invasion of LM whereas the secretion of IL-18 requires LM to gain access to the cytoplasm. The differences with in vivo infection of these different forms of LM illustrate that only live LM can induce an IFN-gamma response. Also, although HKLM can induce IL-12 secretion from macs and DCs in vitro, this is not true in vivo. Perhaps these differences of IFN-gamma and IL-12 secretion in vivo are due to an altered localization of HKLM and LLO-LM in the spleen compared to live LM. We are presently exploring the mechanism by which intracellular localization of live LM is responsible for IL-12 and IL-18 production in vivo. Furthermore, we would like to determine if the anatomical localization of LM infected cells within the spleen is important for IL-12 and IL-18 secretion.

Sponsor: *AI064592, G67700*

909 *(Poster)*

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Classification: Faculty

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REPEATED RESTRAINT STRESS FACILITATES DELAYED LETHALITY, BUT DIMINISHED ACQUIRED RESISTANCE TO PULMONARY PNEUMONIA CORRESPONDING WITH ENHANCED NEUTROPHIL BUT DECREASED DENDRITIC CELL RESPONSIVENESS

Purpose: A link between stress response factors and immune defenses is believed to impact disease outcomes. Dendritic cells (DCs) play an instrumental role in host protection against pathogenic invasion. Thus, further research is needed exploring the effect of stress on antigen presenting cell function. We have established a murine model of repeated restraint stress (RS) for the purpose of investigating cellular immune responses against pulmonary pneumonia. We hypothesize that stress-induced susceptibility to respiratory disease is a result of impaired DC activity.

Methods: Female CD-1 mice were subjected to restraint stress (4 hours per day for 4 days) or no stress. 18 hr following stress, mice were intranasally challenged with S. pneumoniae. In an additional experiment, survivors of the restraint stress group were exposed to a second bout of stress. Both stressed and non-stressed groups were re-challenged with S. pneumoniae. Survival analysis and characterization of cellular immune responses were determined.

Results: Assessment of survival after 36 hr infection revealed that non-stressed mice (NRS) experienced earlier susceptibility to lethality as compared to RS mice, but no difference in overall death. Interestingly, RS survivors subjected to an additional round of stress and infection revealed a significant decrease in survival. Flow cytometry analysis at 18 hr post infection demonstrated that GR1+ CD11b+ cells (neutrophils) were preferentially increased along the respiratory airways as compared to F4/80+ CD11b+ (macrophages) and CD11c+ DCs. Gene array analysis demonstrated preferences in chemokine, but not cytokine expression in the lung of RS mice 3hr post infection. Furthermore, CRH and CRH-receptor 2 mRNA levels were increased in lung tissue.

Conclusions: These data suggest that under conditions of stress, early neutrophil responsiveness mediated by chemokines may be responsible for initial resistance, but a lack of DC and macrophage activity given RS may lead to an inability to mount an effective adaptive immune response against pneumococcal infection.

Sponsor: *N/A*

910 *(Poster)*

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IL-23 ACTS IN A NOVEL FASHION BY ANTAGONIZING IL-12 INDUCED IFN-GAMMA SECRETION

Purpose: Most studies have concluded that interleukin-23 (IL-23) plays a unique role in promoting IL-17 secreting T cells. While some reports have also characterized IL-23 as having redundant pro-inflammatory effects with IL-12, we have instead found that IL-23 can regulate the innate immune response in a novel manner, by antagonizing IL-12 induced secretion of interferon-gamma(IFN-gamma).

Methods: Splenocytes from naive C57BL/6 mice were cultured overnight in complete RPMI medium with varying concentrations of IL-12 and IL-18, or Listeria monocytogenes (LM) infected macrophages, with or without IL-23. IFN-gamma production was determined by ELISA and intracellular cytokine staining with flow cytometry. T cells were purified using a Cytopia Influx cell sorter or BD IMag magnetic bead purification.

Results: Our recent data documents the rapid, innate production of IFN-gamma from memory CD8 T cells, as well as NK cells, stimulated with IL-12 and IL-18. When splenocytes are cultured with IL-23, IFN-gamma secretion in response to IL-12 is dramatically reduced, as measured by ELISA and intracellular cytokine staining. The impact of IL-23 is most prominent in CD8 T cells, but can also be seen in NK, NK-T, and CD4 T cells. We also show that IL-23 can regulate the induction of IFN-gamma by endogenously produced IL-12 from LM infected macrophages. Furthermore, IL-23 appears to act directly on purified CD8 T cells to negatively regulate IFN-gamma induced by IL-12. We are currently investigating the mechanism by which IL-23 inhibits IFN-gamma secretion. Surprisingly, blockade of the IL-23 receptor did not impact the effect of IL-23 on IFN-gamma secretion. The IL-23 receptor is comprised of the IL-12Rbeta1 subunit of the IL-12 receptor and a novel subunit termed IL-23R. This finding suggests that IL-23 may actually be binding the shared IL-12Rbeta1 subunit of the IL-12 receptor. In addition, experiments to determine if IL-23 can inhibit other IL-12 induced effector functions are ongoing.

Conclusions: Our data suggest that IL-23 may be a key player in determining the responsiveness of lymphocytes to IL-12 and their subsequent secretion of IFN-gamma. These experiments further our knowledge of the role of cytokines, particularly IL-23, in immune responses to infectious pathogens. Understanding immune responses to disease causing microbes will enable us to more efficiently control these diseases.

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911 *(Poster)*

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HUMAN NATURAL KILLER CELL RECEPTOR 2B4 (CD244) DOWN-REGULATES ITS OWN EXPRESSION BY OPPOSING PROMOTER ACTIVITIES AT THE AP-1 AND ETS ELEMENTS

Purpose: Natural killer cells are bone-marrow derived lymphocytes that function as key players in innate immunity by recognizing viral, bacterial and parasitic infections and neoplastic target cells. NK cell functions are regulated by specific receptors that, upon interaction with their respective ligands, may send stimulatory or inhibitory signals. 2B4 (CD244), a member of the CD2 subset of the immunoglobulin superfamily, is important for stimulating human natural killer (NK) cell cytotoxicity and cytokine production. It is expressed on all NK cells, a subpopulation of T cells, monocytes, basophils and eosinophils. 2B4 interaction with its ligand CD48 regulates NK, T and B lymphocyte functions and thus plays a central role in various immune responses. We have previously shown that ligation of surface 2B4 on human NK cell line YT induces cytotoxicity, production of IFN-g, matrix metalloproteinases and NK cell invasiveness. Activation protein-1 (AP-1) and Ets were implicated in the transcription of the 2B4 gene. Hence, understanding 2B4 gene expression and regulation will provide further insight into the regulation of immune response during viral infection. In this study we report that stimulation of NK cells through surface 2B4 down-regulates its own expression due to a reduction in the promoter activity at the Ets element and increase in DNA binding activity of AP-1.

Methods: In order to determine that the down regulation of 2B4 was due to a alteration in promoter activity, dual luciferase reporter assays were performed on YT cells after mAb C1.7 stimulation. YT cells were incubated with mAb C1.7 (200 ng/ml) at different time points and total RNA and nuclear extract were obtained. Electrophoretic mobility shift assay (EMSA) was performed with the YT nuclear extract and the labeled probe of human 2B4 promoter. The total RNA from YT cells were reverse transcribed and PCR amplified using gene specific primers. Flow cytometric analyses were also done with the stimulated YT cells.

Results: 2B4 stimulation results in decreased expression of surface 2B4 and 2B4 mRNA. Cross-linking surface 2B4 on YT cells resulted in the increased DNA binding activity of AP-1, whereas it decreased the DNA binding activity of Ets-1.

Conclusions: Our study shows that the down-regulation of 2B4 could be a mechanism to attenuate the co-stimulatory signal from 2B4-CD48 interactions. The down-regulation of 2B4 may be an important factor in controlling lymphocyte activation during immune responses.

Sponsor: *NIH*

912 *(Poster)*

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ANALYSIS OF EXPRESSION OF IMMUNE RECEPTORS IN SUBJECTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Purpose: Systemic lupus erythematosus (SLE) is a female-prone, chronic autoimmune inflammatory disease, characterized by improper regulation of B cells that leads to the production of auto-antibodies. The recently described SLAM family receptors are increasingly recognized, because they take part in immune regulations as well as they can be used to distinguish hematopoietic stem and progenitor cells. Linkage analyses of human chromosome and finding of murine SLE susceptible genes have suggested strong association of SLE with SLAM family receptors, such as NTB-A, 2B4 and CS-1. We hypothesize that the alterations in expression of NTB-A, 2B4, and/or CS1 may mediate the immune dysregulation observed in patients with SLE. The purpose of this study is to compare expression levels of immune receptors expressed on T, B, NK (natural killer) cells, and monocytes in SLE subjects versus those of healthy controls and to analyze the cDNA for 2B4, CS1, and NTB-A at molecular level.

Methods: Blood samples were obtained from subjects diagnosed in SLE and healthy control subjects with prior approval from IRB of UNTHSC and JPS Health Network. After density-gradient centrifugation, peripheral blood mononuclear cells (PBMCs) were stained with fluorochrome-labeled antibodies for CD3, CD19, CD14, CD56, 2B4, CS1 and NTB-A, and analyzed by flow cytometry. Total RNA was isolated from PBMC and analyzed by RT-PCR for 2B4, CS1 and NTB-A.

Results: When compared to healthy control subjects, fractions of CD3-positive T cells and CD56-positive NK cells were relatively low in total PBMC of subjects with SLE, whereas a fraction of CD14-positive monocytes was relatively high in SLE subjects. Some SLE subjects showed decreased 2B4 expression in T cells, NK cells, as well as monocytes. Also, we first found that, like other SLAM related receptors, CS1 and NTB-A are highly expressed on monocytes. This is supported by finding that THP-1 and U937 human monocytic cell lines also highly express CS1 and NTB-A. In RT-PCR for CS1 using total PBMC, two isoforms of CS1-L and CS1-S, as well as other polymorphic forms were differentially detected in SLE subjects.

Conclusions: In subjects with SLE, T lymphocyte fraction and NK cell fraction decreases, in company with an increase of monocyte population, suggesting participation of monocytes in SLE pathogenesis. Since SLAM family receptors 2B4, CS1, and NTB-A were highly detected in monocytes, interaction between monocytes and other immune cells might play an important role in immune regulation.

Sponsor: *UNT-HSC seed grant*

913 *(Poster)*

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TOLL-LIKE RECEPTOR 2 MEDIATES RESPONSES OF ANTIGEN-PRESENTING CELLS IN MURINE RESPIRATORY MYCOPLASMOSIS

Purpose: The purpose of the current study was to determine the Toll-like receptors involved in the recognition of M. pulmonis and their activities during disease progression.

Methods: Pulmonary dendritic cells (DC) and macrophages were isolated from the lungs of infected and naïve C3H/hen mice. Total RNA was isolated and TLR expression was determined via gene microarray and real-time PCR. To assess the protein expression of TLR 2 on dendritic cells and alveolar macrophages, total lung lymphocytes were isolated and subjected to FACS analysis. To determine the physiological function of TLR 2 in vivo, wild-type and TLR 2 KO (C57bl/6) mice were infected 72hrs and cfu were determined for the upper and lower respiratory tract. In addition, bone-marrow derived dendritic cells were generated from wild-type and TLR 2 KO animals and co-cultured in vitro with M. pulmonis whole organism. To determine stimulation, culture supernatants were collected and TNF-a and Il-12 levels were determined using a sandwich ELISA.

Results: We found that TLR 2, 3, 4 and 7 mRNA is expressed in DC and macrophages isolated from the lung of C3H/hen mice. We also found that TLR 2 primary transcript levels change in macrophages and DCs isolated from the lungs of these animals following infection with M. pulmonis. In addition, we observed that TLR 2 protein expression increased on macrophages and dendritic cells following 14day infection with M. pulmonis. Preliminary studies demonstrate that C57bl/6 TLR 2 KO animals have increased cfu in the upper and lower respiratory tract. Also, TLR 2 expression mediates the recognition of the whole organism of M. pulmonis on BMDC stimulated in vitro.

Conclusions: In these studies, we determined that TLR 2 mediates responses to M. pulmonis antigens in vitro. We also determined the TLR mRNA and protein expression changes in pulmonary DC and macrophages following infection.

Sponsor: *N/A*

914 *(Poster)*

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CHARACTERIZATION OF THE IMMUNOPATHOLOGICAL EFFECTS OF MYCOPLASMA PULMONIS MALP-2 HOMOLOGUE MUTANTS

Purpose: As most studies have focused on in vitro interactions between mycoplasma lipoproteins and cells, the impact of lipoproteins in mycoplasma disease progression is not completely understood. While it is clear that M. pulmonis recognition of TLR2 contributes to cell responses, the specific M. pulmonis lipoproteins involved in this interaction are not known. Since MALP-2 is responsible for disease pathogenesis in M. fermentans, this project focuses on examining the possible roles of the MALP-2 homologue in response to M. pulmonis.

Methods: Wild-type and transposon mutant strains of M. pulmonis were obtained. One mutant strain (R4) was used as a control while the other two mutant strains (FC1, FC2) have the MALP-2 homologue gene inactivated. The wild-type strain the mutants were derived from (CT), as well as the M. pulmonis strain used by the lab (WT), also served as controls. For the in vivo studies, BALB/c mice were infected with wild-type and mutant strains, and at 3, 7, and 14 days, the numbers of mycoplasma colony forming units (CFUs) were determine in lungs, spleens, and nasal passages. For the in vitro studies two alveolar macrophage cell lines, J7 and MHS, were infected with mutant and wild-type strains of mycoplasma. The levels of IFN-gamma and IL-6 production were measured using ELISA.

Results: Lung CFU counts for FC1, FC2, and R4 were comparable to WT and CT at day 3. By day 7 FC1, FC2, and R4 lung CFU numbers declined while the WT lung CFU count persisted. At day 14 only the lung WT CFUs persisted. The day 7 and 14 CFU count for CT could not be ascertained due to contamination. In the in vitro studies, IFN-gamma production derived from the J7 cell line was almost undetectable. IL-6 production for all strains was detectable, with greater numbers possibly in R4, FC1, and FC2.

Conclusions: This data indicates differences between the mutants and the wild-types, with R4, FC1, and FC2 lung CFU numbers declining while WT lung CFUs remain high. The mutants may be interacting with the immune system via another route besides TLR2, possibly leading to greater clearance of the organisms than the WT.

Sponsor: *N/A*

1000 *(Poster)*

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POPULATION DYNAMICS AND ASSOCIATED GEOGRAPHIC AND DEMOGRAPHIC FACTORS OF THE DENGUE VECTORS, AEDES AEGYPTI AND AEDES ALBOPICTUS, IN DALLAS COUNTY, TX, USA

Purpose: The potential risk for dengue outbreak was assessed in north central Texas in 2006. The study was conducted in response to increased dengue case numbers in Texas and neighboring Mexican states in 2005 and the growing rate of immigration from dengue-endemic countries into Texas. The abundance of dengue vectors, Aedes aegypti and Aedes albopictus, were assessed in Dallas County, TX from June to October of 2006. Habitat and associated demographic actors were also investigated.

Methods: The abundance parameters of vector oviposition activity was monitored weekly with 108 traps at 54 sites in 6 different zip codes. Oviposition site information was assessed via observation. Demographic and socio-economic information for zip codes were obtained from a public access database, ESRI Sourcebook America, 2005. Efficacy of a common grass-infusion for oviposition traps was also tested.

Results: Dengue vector abundance was highest (46 eggs) in June, decreased to 30 in July and maintained 22-24 eggs after July. Aedes albopictus was more abundant than Aedes aegypti except in October. Aedes albopictus abundance was highest in June and decreased through October. Habitat assessment confirmed that higher vegetation and shade had a higher number of Aedes albopictus and Aedes aegypti ($p<.05$), while standing water also had a higher number of Aedes albopictus ($p<.05$). Analyses also revealed that lower home values corresponded to a higher number of Aedes aegypti ($p<.05$) and homes with lower income corresponded to a higher number of Aedes albopictus. Other socio-economic and demographic factors did not have statistically significant association with dengue vector abundance. Ovitraps with a common grass infusion attracted more eggs than did distilled infusion.

Conclusions: Extreme drought conditions present in 2006 have likely changed typical patterns of Aedes species population dynamics and abundance. Although Aedes aegypti is most abundant in the zip code with highest percent Hispanic population, Hispanic areas in Dallas County may not present a higher risk of dengue outbreak. The dengue mosquito vector is present in Dallas County. A larger scale study should be conducted during a mosquito season with more total precipitation to further investigate the demographic predictors associated with dengue mosquito vector populations.

Sponsor: *Texas Environmental Health Association; Texas Mosquito Control Association*

1001 *(Poster)*

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THE IMPACT OF ALPHA TOXIN ON IN VITRO AND IN VIVO BIOFILM DEVELOPMENT IN STAPHYLOCOCCUS AUREUS

Purpose: Staphylococcus aureus releases several toxins, including alpha toxin, encoded by the gene hla. In addition to its hemolytic activity, recent evidence indicates that alpha toxin plays a role in biofilm formation. We hypothesized that a S. aureus hla knockout (KO) strain (NTH373) would be impaired in its ability to form biofilm under in vitro and in vivo conditions as compared to the wild type parent strain (NTH83).

Methods: In vitro testing was performed using Calgary devices, involving biofilm growth on standard 96 well plates, which were then stained with crystal violet and analyzed for absorbance at 490 nanometers after 48 or 72 hours. Results were confirmed through CFU counts. In vivo testing was performed by growing biofilm on Teflon catheters, which were then implanted subcutaneously into Balb/c mice. Catheters were removed at set time points, and vortexed in phosphate buffered saline (PBS). The solution was then used for spot plating and CFU counts. Additional in vivo testing was performed using a central venous catheter rat model. Jugular cannulated rats were infected intravenously with either wild type or hla KO S. aureus. Catheters and tissues were explanted and plated on days 1 and 3 post-infection.

Results: We found that the hla KO strain forms less biofilm than the wild type strain after 72 hours in vitro. We also showed that mice infected with the hla KO strain displayed less biofilm growth on implanted catheters as compared to wild type infected mice after 7 days in vivo. Finally, we observed that catheters from rats infected with the hla KO strain displayed less biofilm growth after 72 hours as compared to catheters from rats infected with the wild type strain.

Conclusions: In this study, we determined that alpha toxin is involved in biofilm formation under in vitro and in vivo conditions. We observed that hla KO S. aureus was impaired in its ability to adhere to surfaces both in vitro and in vivo as compared to wild type.

Sponsor: *Supported by Cumbre Pharmaceuticals, Inc.*

1100 *(Poster)*

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5-METHOXYDIETHYLTRYPTAMINE SHARES STIMULUS EFFECTS WITH ABUSED HALLUCINOGENS

Purpose: 5-Methoxydiethyltryptamine (5-MeO-DET) is a hallucinogenic structurally related to other tryptamine hallucinogens. It is freely available through internet "pharmacies", but apparently is little used. The behavioral and pharmacological effects not been characterized in laboratory studies.

Methods: The abuse liability of 5-methoxydiethyltryptamine (5-MeO-DET), a non-scheduled hallucinogen, was studied by testing its ability to modify locomotor activity of mice and to produce discriminative stimulus effects in rats similar to the abused hallucinogens, 3,4-methylenedioxymethylamphetamine (MDMA), lysergic acid diethylamine (LSD), (-)-2,5-dimethoxy-4-methylamphetamine (DOM), or dimethyltryptamine (DMT).

Results: In doses from 0.3 to 10 mg/kg, 5-MeO-DET produced a dose-dependent but mild (60% of methamphetamine) stimulation of locomotor activity that occurred after 20 minutes and lasted 80 to 90 minutes. Locomotor depressant effects occurred during the first 30 min after 10 mg/kg of 5-MeO-DET. 5-MeO-DET (ED50= 0.38 mg/kg) substituted fully only in rats trained to discriminate DMT (5 mg/kg), but substituted partially (39-59% drug-appropriate responding following 0.5 to 2.5 mg/kg) in rats trained to discriminate DOM (0.5 mg/kg) or MDMA (1.5 mg/kg). 5-MeO-DET (0.025 to 5 mg/kg) produced little or no substitution in rats trained to discriminate LSD (0.1 mg/kg), methamphetamine (1 mg/kg), or cocaine (10 mg/kg). 5-MeO-DET resulted in dose-dependent decreases in response rate in the dose range of 1 to 5 mg/kg, with some variability in potency for this effect among the groups of rats trained to different drug stimuli. Tremors (2/3 rats) and lethality (1/3 rats) were observed following 5 mg/kg 5-MeO-DET.

Conclusions: Due to the apparent similarity of its discriminative stimulus effects to DMT, a known drug of abuse, 5-MeO-DET may itself have potential for abuse. The presence of adverse effects at doses close to those substituting for DMT suggests, however, that abuse of 5-MeO-DET could be relatively more hazardous. It is noteworthy that training of the discriminative stimulus effects of DMT has not previously been reported, yet the apparent selectivity observed in the current studies may indicate that this hallucinogen represents an important standard for abuse liability testing.

Sponsor: *NIDA*

1101 *(Poster)*

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UNCOUPLING AXON PATHOLOGY FROM INFLAMMATION IN A MODEL OF EARLY AUTOIMMUNE OPTIC NEURITIS

Purpose: Discovery of treatments to protect axons and prevent permanent disability associated with progressive MS has faced the up-hill challenge of assessing relatively small changes in accumulated axon damage within a background environment that is profoundly disorganized by CNS inflammation. These studies address that problem. HYPOTHESIS: We hypothesized that early transient treatment with 5-(4-phenylbutoxy)psoralen (PAP-1), a treatment previously shown to suppress effector memory T-cell proliferation, would suppress autoimmune CNS inflammation.

Methods: A model of autoimmune optic neuritis was transiently treated (days 3-8 after antigen exposure) with PAP-1.

Results: Thirteen days after antigen exposure, samples were assayed for MS-like pathologies using microfluorimetry. Markers of neuroinflammation and immune infiltration were effectively suppressed ($p<0.05$) by transient PAP-1 treatment, often to levels below those found in normal nerve. Markers of MS-like axon pathology were not suppressed, and showed increased severity compared to vehicle-treated autoimmune nerve ($p<0.05$).

Conclusions: Transient treatment with PAP-1 functionally uncoupled development of MS-like axon pathology from development of CNS inflammation in a model of autoimmune optic neuritis, consistent with a very early branching of the underlying biochemical pathway.

Sponsor: *NIH/NEI*

1102 *(Poster)*

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ACTIVATION OF A MEMBRANE ANDROGEN RECEPTOR PROMOTES CELL DEATH IN GLIA

Purpose: Androgens, such as dihydrotestosterone (DHT), are involved in numerous biological functions including the regulation of cell viability. However, conflicting reports exist as to whether androgens are protective or damage-inducing. Since androgens may elicit their effects through activation of the "classical" androgen receptor (AR) or alternatively, through a putative plasma membrane receptor, we proposed that this discrepancy may be attributed to differential activation of these two mechanisms. To test this hypothesis, we evaluated whether activation of the putative membrane AR in glia, suppresses the activity of survival pathways and increases in cell death during injury, relative to the effects mediated through the classical (intracellular) AR.

Methods: Primary cortical astrocytes from mice were isolated and plated on postnatal day 3 (P3) and treated on day 10 in vitro (DIV). The primaries were maintained in DMEM supplemented with 10% serum and the cells were treated when the cells were 90% confluent. The cultures were treated with iodoacetic acid (IAA; 10 ?M) in the presence or absence of DHT (10 ?M) or DHT-BSA (membrane impermeable form of DHT; 10 ?M) for 12 hours. Indices of cell death included measurements of lactate dehydrogenase (LDH) release into the media, caspase-3/7 activity, and TUNEL staining. In parallel, we assessed the phosphorylation of Akt, an important effector of the protection associated with the PI-3 kinase pathway.

Results: In the primary cortical astrocytes, DHT protected against IAA-induced toxicity. In contrast, DHT-BSA treatment exacerbated IAA-induced toxicity. This effect was flutamide (intracellular AR antagonist) insensitive, suggesting that the mAR is distinct from the intracellular AR. In addition, activation of the mAR, led to a decrease in phospho-Akt levels and a correlative increase in caspase-3/7 activity and TUNEL staining. Interestingly, the damage promoting consequences of activating the mAR influenced other steroid hormones ability to protect, since DHT-BSA blocked estrogen-induced protection.

Conclusions: Collectively, these studies indicate that activation of the mAR during injury, leads to an increase in glial cell death. In contrast, activation of the intracellular AR protects. Thus, depending on the predominance of one receptor mechanism over another, the outcome of androgen treatment may be very different, and as such, could help explain existing discrepancies as to whether androgens are protective or damage-inducing.

Sponsor: *N/A*

1103 *(Poster)*

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THE ROLE OF CRITICAL AMINO ACID RESIDUES IN MOUSE AND HUMAN 5-HT3A RECEPTORS IN CONFERRING DIFFERENTIAL PHARMACOLOGICAL SENSITIVITIES TO 2-OHMBA

Purpose: Use 2-OHMBA and its differential effects on human and mouse 5HT3A receptors to refine our understanding of the ligand binding domain and the amino acids that play a role in gating and function of the receptor.

Methods: Oocytes were microinjected in the cytoplasm with wild type and mutant 5-HT3A-R cRNA transcribed in vitro. Recording was done from days 3-6 following injection in a 100 ul volume recording chamber, and oocytes were voltage clamped at -70mV. Currents were plotted on a strip chart recorder.

Results: Mouse receptors containing mutations at I207, with the exception of Leucine, resulted in a loss of partial agonist activity and a gain in antagonist activity in response to 2-OHMBA.

Conclusions: Loop C and Loop F of mouse and human 5-HT3A receptor account for differential pharmacology. Discrete physicochemical properties are required for gating of the mouse 5-HT3A receptor.

Sponsor: *N/A*

1104 *(Poster)*

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EFFECTS OF 17BETA-ESTRADIOL ON FOCAL CEREBRAL ISCHEMIA INDUCED BY EMBOLIC MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS

Purpose: This study was designed to determine whether E2 exerts neuroprotective effects in an embolic MCAO rat model, which closely resembles clinical embolic stroke. Further, we investigated whether E2 treatment extends the therapeutic time window of recombinant tissue plasminogen activator (rt-PA) in this model.

Methods: Focal cerebral arterial ischemia stroke was induced by injection of clots into the internal carotid artery (ICA) to occlude the middle cerebral artery (MCAO) in ovariectomized (OVX) female rats. To investigate the neuroprotective effects of E2 on ischemic stroke induced by embolic MCAO, the rats were divided into three groups: control (CRTL), E2 pellet treatment, and E2 acute treatment. In CRTL group, a 3cm long corn oil pellet was implanted subcutaneously for a week. In the E2 pellet group, a 3cm long pellet contains 4mg/ml E2 was implanted subcutaneously for a week. In the E2 acute group, 100μg/ml E2 was administered (100mg/kg, s.c.) 2 hours before ischemia. To determine whether E2 treatment extends therapeutic time window of rt-PA, the rats were subjected to embolic infarction and assighed into three groups: early rt-PA treatment group, at which rtPA was administered at 0.5hr after embolization; later rt-PA treatment group, at which rt-PA was administered at 3hr after embolization; and combined rt-PA and E2 group, at which E2 was administered immediately after embolization and rt-PA was administered at 3hr after. Animals were sacrificed at 24 hours after embolization, brains were harvested and processed for lesion volume analysis by 2,3,5-triphenyltetrozolium chloride (TTC) staining.

Results: Lesion volume was 284.9 ± 50.28mm3 (n=9), 96.58 ± 27.02 mm3 (n=9) and 274.8 ± 65.30 mm3 (n=7) in CRTL, E2 pellet, and E2 acute group, respectively. Chronic treatment with E2 significantly decreases mortality and ischemic lesion volume ($p<0.01$). No significant difference in lesion volume and mortality rate was indicated between CRTL and E2 acute treatment group ($p>0.05$). On the other hand, acute treatment of E2 significantly extends the therapeutic window of rt-PA beyond 3hr ($p<0.05$).

Conclusions: Our results suggest that 17ß-estradiol (E2) exerts neuroprotective effects in the embolic middle cerebral artery occlusion (MCAO) rat model when administered subcutaneously a week before ischemic insult. Furthermore, acute treatment of E2 extends the therapeutic time windows for thrombolysis.

Sponsor: *NIH Grants AG 10485 and AG22550*

1105 *(Poster)*

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INHIBITION OF C-JUN NH2-TERMINAL KINASES (JNKS) REPRESSES THE EXPRESSION OF PRESENILIN 1

Purpose: Ets1 and Ets2 bind to the -10 Ets site of the PS1 promoter and activate PS1 transcription. p53 negatively regulates PS1 transcription without interacting with the PS1 promoter. Protein kinase pathways are major intracellular signaling pathways that regulate expression of many genes. We studied the effects of different protein kinase inhibitors on PS1 gene expression and identified that c-jun-NH2-termal kinases (JNKs) play important roles in the repression of PS1 transcription by p53. This study will provide groundwork to dissect the relationship between the general cell signaling transduction pathways and the pathogenic features of PS1.

Methods: -Chromatin-immunoprecipitation (ChIP) to identify in vivo DNA-protein interaction between the PS1 promoter and Ets1/Ets2 transcription factors. -Chloramphenicol acetyl transferase (CAT) reporter assay to monitor PS1 promoter activity -Immunoblot assay to determine the expression of phosphor-JNK, p53, c-jun, PS1, and Actin. -Reverse transcriptase polymerase chain reaction (RT-PCR) to determine PS1 mRNA level -Co-immunoprecipitation (Co-IP) to determine the interaction between p53 and Ets1/Ets2

Results: Pharmacological inhibition of PI3K kinase, MEK kinase, ERK kinase failed to induce significant changes in presenilin-1 (PS1) expression in SK-N-SH neuroblastoma cell line. JNK inhibitor SP600125 significantly increased p53 protein level and decreased PS1 mRNA and PS1 protein expression in SK-N-SH cells. ChIP assay showed that Ets1/Ets2 binding to the PS1 promoter is decreased by JNK inhibitor without any change of p53 association into the PS1 promoter. Furthermore, co-immunoprecipitation study showed that the interaction of p53 with Ets1/Ets2 was increased by JNK inhibitor. These data suggest that activation of p53 by JNK inhibitor decreased the DNA binding ability of Ets1/Ets2 into the PS1 promoter resulting in the repression of PS1 expression. In fact, overexpression of p53 in p53-deficient PC3 cells efficiently inhibits the DNA binding of Ets1/Ets2 into the PS1 promoter region and also represses PS1 expression.

Conclusions: We found that inhibition of JNKs using pharmacological inhibitor decreases expression of PS1 by increasing the interaction between p53 and Ets1/Ets2 transcription factor. Furthermore, we found that p53 interferes with the DNA binding ability of Ets1/Ets2, an activator of PS1 transcription. In conclusion, our data suggest that JNK regulates PS1 expression through protein-protein interaction between p53 and Ets1/Ets2.

Sponsor: *NIH grant R01AG18452-04*

1106 *(Poster)*

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AGE EXACERBATES THE EFFECTS OF ETHANOL WITHDRAWAL ON OXIDATIVE DAMAGE TO PROTEINS IN AN ESTROGEN REVERSIBLE MANNER

Purpose: We have demonstrated that abrupt termination of chronic ethanol diet creates lipid peroxidation in young adult rats in a manner protected by endogenous estrogen [(17ß-estradiol (E2)].

Methods: In this study, we tested a deleterious interaction between ethanol withdrawal (EW) and age at the levels of protein oxidation and E2 protection. 5-, 12-, and 16-month-old ovariectomized rats with oil pellet or acute E2 replacement received a liquid ethanol (6.5%) or control dextrin diet for 2 weeks. After testing for EW signs at 24 hours of EW, they were immediately sacrificed. Cerebellum, cortex, hippocampus were collected to assess the levels of carbonyl as a marker of oxidative damage to protein.

Results: As compared to the control dextrin, EW increased the levels of carbonyl in three brain areas with a greatest damage to cerebellum. The EW-induced increase in the levels of carbonyl was more prominent in 16-month-old rats than 5-month-old rats. E2-treatment lowered the levels of carbonyl in EW rats but such effects of E2 were more prominent in 5-month-old rats than 12- or 16-month-old rats. In addition, there was a high correlation between EW sign scores and the levels of carbonyl in three age groups.

Conclusions: These findings suggest that age exacerbates EW-induced oxidative damage to proteins in an age and brain region specific manner. Estrogen protection against the deleterious interaction between age and EW appears to be more efficient in younger rats than old rats.

Sponsor: *NIAAA AA013864 and AA015982*

1107 *(Poster)*

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POSTNATAL MATURATION OF DIHYDROLIPOAMIDE DEHYDROGENASE IN RAT BRAIN

Purpose: Mammalian mitochondrial dihydrolipoamide dehydrogenase (DLDH) is a stable homodimeric FAD-dependent disulfide oxidoreductase. In vivo, DLDH catalyzes the oxidation of dihydrolipoamide at the expense of NAD+. In vitro, however, DLDH can act as a diaphorase by transferring electrons from NADH to artificial electron acceptors such as nitroblue tetrazolium (NBT) and 2,6-dichlorophenolindophenol (DCPIP). The objective of this study was to investigate the mechanisms by which DLDH undergoes postnatal maturation in brain mitochondria using rat as a model.

Methods: Three groups of rats, aged 21 days, 5 months or 30 months were used. DLDH activity was measured by four different assays, which included a forward reaction measuring dihydrolipoamide oxidation by NAD+, a reverse reaction measuring lipoamide reduction by NADH, a gel-based histochemical staining method measuring DLDH diaphorase activity using the NBT/NADH detection system, and, a colorimetric measurement of DLDH diaphorase activity by the DCPIP/NADH method. All assays were performed using mitochondrial extracts as the enzyme source.

Results: DLDH diaphorase activity assay showed no detectable differences among the three age groups; however, the level of DLDH dehydrogenase activity was significantly lower in 21-day-old rats when compared to 5- or 30-month-old rats. Additionally, no difference on the level of DLDH dehydrogenase activity was observed between 5- and 30-month old rats. The level of dehydrogenase activity for 21 day-old rat was determined to be similar in both the forward reaction and the reverse reaction.

Conclusions: The results of this study indicate that the dihydrolipoamide binding domain, but not the NADH binding domain, is involved in DLDH postnatal maturation. This study suggests that the region where dihydrolipoamide binds could be potentially susceptible to post-translational oxidative modifications under oxidative stress conditions.

Sponsor: *National Institutes of Health*

1108 *(Poster)*

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NEUROPROTECTIVE EFFECT OF PALMITOYLETHANOLAMIDE AGAINST TRANSIENT CEREBRAL ISCHEMIA

Purpose: Cerebral ischemia is the third largest cause of death among humans in developed countries. The common cause of cerebral ischemia is the interruption of blood supply to the brain. Cannabinoids have been proposed as treatment for a wide spectrum of medical disorders including stroke. Anandamide is an endogenous cannabinoid receptor ligand, which protects cortical neurons against hypoxia and glucose deprivation in in vitro models. Palmitoylethanolamide (NAE 16:0) is a structural analogue of anandamide. The present study was conducted to determine effects of NAE 16:0 in an in vivo model of transient cerebral ischemia.

Methods: Three experimental groups were used in this study, a control group (vehicle), an ischemic group (vehicle + ischemia) and a treated ischemic group (NAE 16:0 + ischemia). NAE 16:0 was administered 6h before and then again at 30min before ischemia onset. Ischemia was induced for 90 min followed by a 24 hr reperfusion period. After assessing neurobehavioral activity, effects and extent of ischemia as well as neuroprotection by NAE 16:0 were measured with histological methods and determined using image analysis software (SimplePCI v.6.2, Compix Inc., Hamamatsu Photonics Management, Sewickley, PA).

Results: NAE 16:0 treatment showed neuroprotection by significantly decreasing the extent of ischemia (11.3% ± 2.6) when compared to the vehicle treated ischemic group (38.4% ± 5.7) ($p < 0.01$; treated versus ischemia). Neuroprotection was also reflected in the neurobehavioral activity of NAE 16:0 treated ischemic versus vehicle treated ischemic groups. Neurological deficit scores were significantly improved in the treated group (0.6 ± 0.3) as compared to the ischemic group (2.3 ± 0.3) on a 0-3 scale ($p < 0.01$; treated versus ischemia).

Conclusions: The present study demonstrates for the first time the neuroprotective effect of NAE 16:0 in transient cerebral ischemia. NAE 16:0 may have therapeutic potential in disorders resulting from ischemia and potentially in other neurodegenerative disorders by protecting neurons and/or maintaining their viability.

Sponsor: *NIH/NIA*

1109 *(Oral)*

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Presenter: Ivan Lee

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Classification: GSBS Student

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CHARACTERIZATION OF SIGMA-1 RECEPTOR LIGANDS USING AN UNAMBIGUOUS ASSAY

Purpose: Sigma-1 receptors have been implicated in playing a possible role in the etiology and therapy of a wide range of cognitive, addictive and neurological disorders. These range from the development of new antiamnesics to the treatment of Alzheimer's disease and traumatic injuries. These important potential implications in medical treatments call for a better knowledge of the relationship between the Sigma-1 receptor and its ligands. However, a wide range of affinity values for various Sigma-1 receptor ligands have been reported in whole tissues, making it difficult to ascertain true values for this receptor. The purpose of this study was to determine unambiguously the affinity of potential ligands for the Sigma-1 receptor.

Methods: The cloned human Sigma-1 receptor was transfected into human breast adenocarcinoma (MCF-7) cells, which do not express drug-sensitive Sigma-1 receptors. Individual clones were established over a period of weeks on the basis of G418 selection. Saturation radioligand binding was used to determine receptor expression levels in the stable cell lines. Affinity values for potential ligands were measured via a radioligand competition binding assay using [3H](+)-pentazocine.

Results: The Sigma-1 radioligand [3H](+)-pentazocine binds with a high affinity (KD (s.d.) = 3.7 ± 0.87 nM) to a high density (Bmax (s.d.) = 109 ± 23.7 pmol/mg) of receptors in cells stably transfected with the human Sigma-1 receptor, but not in untransfected cells. Known Sigma-1 receptor ligands, haloperidol and BD1063, bind with the expected low nanomolar affinities, and the stereoisomers of SKF-10047 have the expected selectivities. Utilizing this reliable assay system, the unambiguous affinity values (Ki) for the Sigma-1 receptor were determined for approximately two dozen neurosteroids, benzomorphans, butyrophenones, D4 dopamine receptor-selective ligands, various typical and atypical antipsychotic drugs, and drugs of abuse.

Conclusions: In addition to explaining some noteworthy findings in previous reports, these results supported some, but not all prior Sigma-1 receptor ligand affinity studies utilizing whole tissue models.

Sponsor: *G67673 (JAS) and G67710*

1110 *(Poster)*

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HIPPOCAMPAL NEURONS LACKING HOMER-1A BUT NOT HOMER-1B ARE MORE SUSCEPTIBLE TO GLUTAMATE-INDUCED OXIDATIVE STRESS AND CELL DEATH

Purpose: Complex brain functions, such as learning and memory, involve changes in synaptic strength the efficiency of communication between nerve cells. At the postsynaptic membrane of excitatory synapses, neurotransmitter receptors are clustered to a large protein signaling complex within the postsynaptic density (PSD) that regulates the strength of synaptic transmission. The Homer protein family has been implicated in the regulation of synaptic strength because of its clustering activity recruiting neurotransmitter receptors found at the PSD. Homer proteins regulate transient receptor potential channels, intracellular calcium channels, and other scaffolding protein, such as Shank the PSD. Specifically, Homer 1 interacts with several receptors such as metabotropic glutamate receptors at the plasma membrane and IP3 receptors on the endoplasmic reticulum membrane to regulate the intracellular calcium concentration and calcium dependent functions. The purpose of these experiments was to investigate the complex interactions of Homer 1 at the synaptic level. Data from this experiment will provide information for the mechanisms of calcium mediated neuroprotection in the CNS. We tested the hypothesis that the knockout of Homer 1a leads to a decreased cell viability and higher rate of apoptosis when cells experience glutamate-insult.

Methods: We used cultured Homer 1a KO hippocampal neurons to measure cell viability and apoptosis with Cyto Tox-ONE Homogeneous Membrane Integrity (measures Lactate Dehydrogenase (LDH)) and DeadEnd Fluorometric TUNEL System assays were performed after treatment with glutamate.

Results: Increased numbers of TUNEL positive cells stain in the Homer 1a KO group when compared to the +/- Homer 1a KO group (n=6, F = 0.0653). The LDH assay corroborated this result in that there was measured a significant increase in LDH release when compared to controls (n=7, p?0.001).

Conclusions: These results indicate that the protein Homer 1a potentially plays a significant role in the regulation of intracellular signaling and neurodegeneration. In summary, the data from these experiments indicate that the lack of Homer 1a protein causes increased cell death. This data also supports the notion that the dominant negative protein, Homer 1a, promotes neuroprotection when cells experience oxidative stress.

Sponsor: *National Institute of Aging*

1111 *(Poster)*

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Department: Pharmacology & Neuroscience

Classification: GSBS Student

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REGULATION OF THE GABA-A RECEPTOR BY THE ERK/MAPK PATHWAY

Purpose: The GABA-A receptor is a ligand-gated chloride channel whose function can be modulated by phosphorylation of its various subunits. We found a consensus phosphorylation sequence for ERK, a key effecter of the MAPK pathway, within the intracellular loop of almost all α subunits of the GABA-A receptor. Thus, we hypothesized that the α subunits of the GABA-A receptor may be important targets of the ERK/MAPK pathway and the phosphorylation of the α subunits may alter the function of the GABA-A receptor.

Methods: We tested our hypothesis in α1β2γ2-stably transfected HEK 293 cells and HEK293 cells that were transiently transfected with a α1 subunit in which the putative ERK phosphorylation site, threonine (T) 375, was mutated to alanine (A). GABA-A receptor function was assessed using conventional whole cell and perforated patch clamp electrophysiology in the presence or absence of a pharmacological inhibitor of ERK, U0126, and an activator, hepatocyte growth factor (HGF). The effect of U0126 and HGF on ERK phosphorylation was confirmed by Western blot.

Results: Inhibition of the ERK/MAPK pathway reduced basal ERK phosphorylation, and resulted in an enhancement of GABA-induced peak current amplitudes in α1β2γ2-transfected HEK293 cells, an effect seen only with the perforated patch clamp methodology, while conventional whole cell patch showed no effect of the pharmacological inhibitor. Mutation of the ERK phosphorylation site not only prevented the U0126-induced enhancement of GABA-gated currents, but also resulted in U0126-mediated inhibition of GABA-A receptor function. Activation of the ERK/MAPK pathway with HGF did not show any effect in conventional whole cell patch.

Conclusions: Our data demonstrated the presence of a novel mechanism for regulating the GABA-A receptor through the ERK/MAPK pathway. Given that U0126 was only able to elicit an effect on GABA-gated currents under perforated patch clamp conditions but not under conventional whole cell patch conditions supports the requirement of an intact intracellular milieu. Based on the mutant results, we suggest that the putative ERK phosphorylation site, T375, is involved in the ERK/MAPK-mediated receptor regulation although there might be sites other than T375 that serve as an important target of ERK in regulating GABA-A receptor function.

Sponsor: *N/A*

1112 *(Poster)*

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ESTROGENS DIRECTLY POTENTIATE NEURONAL L-TYPE CALCIUM CHANNEL

Purpose: To determine the molecular mechanism by which estroge induces intracellular calcium influx in neurons

Methods: measured L-type voltage gated calcium currentin hippocampal neurons by patch clamp method. measured intracellular calcium influx by fura2 dye imaging method.Transfection of voltage gated L-type subunits into HEK293 cells

Results: estrogen potentiates L-type voltage gated calcium channel in a dose dependent manner and the action of estrogen does not require estrogen receptors.

Conclusions: estrogen by activating L-typecalcium channel induces intracellular calcium influx in hippocampal neurons.

Sponsor: *nih*

1113 *(Poster)*

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Presenter: J. David Orr

Department: Internal Medicine

Classification: Faculty

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"STILL'S SIGN": THE CASE FOR A NEW EPONYM IN CLINICAL MEDICINE

Purpose: To make the case for a new eponym in clinical medicine designated as "Still's sign," to memorialize the observation by Dr. Andrew Taylor Still in 1838 that compression of the extracranial arteries is associated with pain relief during migraine headache.

Methods: A literature review with the search terms: migraine, physical signs, history of medicine, history of headache/migraine, medical eponyms, Andrew Taylor Still, and medical quotations was performed using MEDLINE (1950-2006), OSTMED, Medicine in Quotations Online ACP, www. Whonamedit.com, National Library of Medicine NIH, and The Still National Osteopathic Museum and Archives.

Results: The venerable tradition of naming medical phenomena after persons associated with their practice or discovery is over 2500 years old. There are over 7500 medical eponyms that range from "Hippocratic facies," to the "Babinski sign." Usage in the medical literature confirms application of eponyms. Currently, there is no designation called, "Still's sign." Dr. A.T. Still in 1898 and 1904 (Journal of Osteopathy), and in 1908 (Autobiography) recorded his original observations regarding a technique which treated migraine headaches by compression of extracranial arteries. The current theory of migraine invokes neurovascular pathophysiology and sterile inflammation of extracranial arteries. The initial descriptions of this by Dr. Harold Wolff in his seminal text (Headache) in 1963 included the very same observation regarding compression of the extracranial arteries and relief of migraine headache.

Conclusions: Based on the doctrine of prior publication, and the fact that A.T. Still founded his science of Osteopathy on the original observation that compression of the extracranial arteries during migraine leads to relief of pain, we are making the case for a new eponym in clinical medicine called, "Still's sign," to memorialize the efforts of a great pioneer in American medicine.

Sponsor: *N/A*

1114 *(Poster)*

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Presenter: J. David Orr

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POST-STROKE CENTRAL PAIN RESPONDS TO NOVEL ANTICONVULSANTS

Purpose: Chronic neuropathic pain is a commonly encountered clinical problem. Multiple trials have shown the relative efficacy of antiepileptic drugs (AEDs) in this setting. However, there are no trials which report the effects of AEDs in the syndrome of Central Post-Stroke Pain (CPSP), the thalamic pain syndrome of Dejerine-Roussy. This report documents the efficacy of the novel class of AEDs called alpha-2-delta ligands at voltage-gated calcium channels in CPSP in a prospective series of 3 patients.

Methods: A case series of 3 patients diagnosed with CPSP and verified with MRI were studied prospectively using a Likert pain scale. They had been previously treated with multiple pain medications including NSAIDs, opiates, tricyclic antidepressants (TCA), muscle relaxants, and prior AEDs. The novel AED alpha-2-delta ligands, gabapentin and pregabalin, were titrated to efficacy. A review of the literature for CPSP, neuropathic pain, and central pain syndromes included MEDLINE (1966-2006), and the Cochrane Database for systematic reviews.

Results: All patients had CPSP based on clinical presentation and confirmed with MRI. The initial Likert pain score was 10 (0-10) in each case, despite treatment with multiple prior medications. With initiation and titration of the alpha-2-delta ligand AEDs the pain scores improved 50-73% in all cases. No serious adverse effects were noted. The literature review found no case series with 3 or more patients treated for CPSP with the alpha-2-delta ligands pregabablin and gabapentin. No RCT exists with any AEDs for CPSP. The average Number Needed to Treat (NNT) for gabapentin in chronic neuropathic pain from RCTs is 4.3. Generally, 40-60% of patients with chronic neuropathic pain will respond to these agents.

Conclusions: This report represents the first prospective series of 3 patients diagnosed with CPSP, verified with MRI, and treated with alpha-2-delta ligands. We found 100% response rates with pain scores decreasing 50-73% for over 6 months of follow-up. The putative mechanism of gabapentin and pregabalin as alpha-2-delta ligands blocking voltage-gated calcium channels is consistent with findings that such blockade results in decreased glutamatergic activity. This suggests a prominent role for disinhibition of thalamo-cortical circuits in the generation of central pain syndromes.

Sponsor: *N/A*

1115 *(Poster)*

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Classification: GSBS Student

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ESTROGEN-DERIVED PARA-QUINOLS LACK FEMINIZING EFFECTS IN OVARIECTOMIZED RATS AFTER CHRONIC TREATMENT

Purpose: Estrogens are well documented as neuroprotective agents, however the side effects of feminizing estrogens can be undesirable and even detrimental. We have demonstrated an antioxidant cycle of estrogens involving para-quinol intermediates that may be exploited as a non-feminizing prodrug of the hormone. An acute dose of the estrone-quinol 2 hours prior to surgery is equipotent with its parent in reducing lesion volumes in a transient cerebral occlusion mode of stroke but was found to be non-feminizing in the estrogen-sensitive uterus. Because non-feminizing estrogenic compounds are desired but not currently available, we assessed the effects of chronic administration of para-quinols derived from estrone and 17ß-estradiol on estrogen-responsive tissues in ovariectomized rats to determine if this compound exerts feminizing effects.

Methods: Three groups of ovariectomized rats received subcutaneous injections of estrone, estrone para-quinol (100µg/kg body weight), or vehicle every 48 hours for a period of two weeks. Another three groups received injections of estradiol, estradiol para-quinol (100µg/kg body weight), or vehicle. Forty-eight hours after the final injection, the rats were sacrificed and the uteri and anterior pituitary glands were collected and weighed.

Results: Wet uterine weights of the estrone group were nearly three-fold higher than those of the quinol group, but there were no statistically significant differences among the groups in anterior pituitary weights (albeit an increase in the estrone group was apparent). At the end of the treatment period, the estrone quinol group also had significantly higher body weights than the estrone group. We also confirmed by experiments probing in vitro that reduction of estrone-derived para-quinol to the parent estrone was nearly 200-times faster in brain tissue compared to uterus homogenate. Wet uterine weights of the estradiol group were also approximately three-fold higher than those of the quinol group; however there was a significant increase in the anterior pituitary weight in the estradiol group compared to the vehicle and quinol groups.

Conclusions: Collectively, these data indicate that even chronic administration of para-quinols does not result in feminization, most probably due to its limited conversion to the parent estrogen in the uterus. However, this conversion may occur readily in tissues not involved in the endocrine action of the hormone, such in the suprahypothalamic regions of the brain.

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1116 *(Oral)*

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MODEL SYSTEMS OF NEURODEGENERATION EXPRESS PROTEINS INVOLVED IN N-ACYLETHANOLAMINE SIGNALING

Purpose: The purpose of this study was to test the hypothesis that cultured primary cortical neurons and the murine hippocampal cell line HT22 express proteins involved in N-acylethanolamine (NAE) signaling. NAEs are lipids found in most tissues including neurons and are upregulated in response to cell and tissue injury. Furthermore, NAEs exhibit neuroprotective properties in in vivo and in vitro models of disease. Some NAEs such as anandamide, an endocannabinoid, exert most of their actions through the G-protein-coupled cannabinoid receptors (CB1 or CB2), but other targets of non-cannabinoid receptor-binding NAEs are not fully understood. The significance of this study is that detection of NAE signaling proteins in HT22 cells and cultured cortical neurons make these model systems useful for studying the neuroprotective mechanisms of NAEs.

Methods: We utilized immunocytochemistry and western blotting to detect the presence of NAE signaling proteins.

Results: We determined that cultured cortical neurons and HT22 cells express proteins involved in NAE function including both types of cannabinoid receptors, the transient receptor potential V1 receptor (TRPV1), and the NAE-degrading enzyme fatty acid amide hydrolase (FAAH).

Conclusions: We conclude that cultured cortical neurons and HT22 cells will be useful cell culture model systems for studying the neuroprotective effects of NAEs and pharmacological inhibitors of NAE signaling proteins.

Sponsor: *NIH/NIA*

1117 *(Poster)*

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Presenter: Andrew Wilson

Department: Pharmacology & Neuroscience

Classification: Staff

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THE EFFECTS OF HYPOXIA CONDITIONING ON ETHANOL WITHDRAWAL IN AN IN VITRO MODEL

Purpose: We have shown that ethanol withdrawal toxicity is more sever than ethanol exposure in rats and showed a similar effect in a cellular model of ethanol withdrawal that could be developed in a cultured hippocampal cell line (HT22). Hypoxia is the reduction of oxygen in tissues, below levels considered to be normal. Intermittent hypoxia Conditioning (IHC) is hypoxia subjected to interruption or periodic stopping. IHC has been used to treat and prevent a variety of diseases, to increase the efficiency of exercise training, and protect against ischemia. Chronic hypoxia may have serious pathophysiological effects however, hypoxia may be tolerated and produce favorable effects if duration is brief or reduction in inspired gas is more moderate. The ultimate balance between excessive production of free radicals and enhancement of Antioxidative processes directly depends on the experiment regime.

Methods: HT22 cells were exposed to ethanol for a 24h period in the presence and absence of IHC. The ethanol solution was then removed from the cells for 4 h to create ethanol withdrawal. Samples were collected at the end of a 24-h ethanol exposure or at 4h of ethanol withdrawal to assess cell viability using a calcein assay, lipid peroxidation by measuring malondialdehyde, and protein oxidation by measuring carbonyl contents.

Results: When tested, ethanol concentrations were constantly maintained during a 24-h ethanol exposure and eliminated at 4 h of ethanol withdrawal. Ethanol withdrawal decreased cell viability and increased the levels of malondialdehyde and carbonyls more than ethanol exposure. IHC reduced the cell death and malondialdehyde levels while the increased carbonyl contents were reduced only by IHC treatment.

Conclusions: These data suggest that ethanol withdrawal can be created in HT22 cells in a manner that is more toxic than ethanol exposure and that IHC can be protective against cell death and oxidative damage induced by ethanol withdrawal. Therefore, IHC can be a potential therapy for a cellular and oxidative imbalance associated with ethanol withdrawal.

Sponsor: *N/A*

1118 *(Poster)*

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PHOSPHORYLATION OF SYNAPTIC PLASTICITY SPECIFIC PROTEINS BY ESTROGEN

Purpose: To determine if estrogen's synaptic action is involved in the temporal regulation of the phosphorylation of alpha-CaMKII and CREB.

Methods: Primary rat cortical and hippocampal neurons treated with estradiol (10nM) were used for western blot and immunofluorescence analysis respectively. Phosphospecific antibody was used to detect the activation via phosphorylation of alpha-CaMKII and CREB.

Results: Estradiol treatment (10nM) induces the activation via phosphorylation of alpha-CaMKII and CREB. This effect is elicited in primary rat cortical and hippocampal neurons using two different methodologies.

Conclusions: These data suggest that estrogen, via phosphorylation of alpha-CaMKII and CREB, is in part the signaling mechanism by which estrogen contributes to learning, memory formation, and LTP.

Sponsor: *NIH grants AG10485 and AgG22550*

1119 *(Poster)*

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EVIDENCE FOR INTERPLAY BETWEEN TRH AND ITS STRUCTURAL ANALOGUE [GLU2]TRH IN THE BRAIN

Purpose: Thyrotropin-releasing hormone (TRH; pGlu-His-Pro-NH2) and [Glu2]TRH (pGlu-Glu-Pro-NH2) are structurally related endogenous peptides that are identified in neuronal and non-neuronal tissues. The purpose of our study was to demonstrate the link between neuropharmacological activities exerted by [Glu2]TRH and potential stimulation of ACh release in the mammalian brain. TRH and its analogues have been considered lead compounds for the development of agents potentially useful for the treatment of several CNS diseases including Alzheimer's disease, amyotrophic lateral sclerosis, epilepsy, mood disorders and depression.

Methods: We selected intracerebral microdialysis in the hippocampus of rats for the in vivo testing of our study's hypothesis, because TRH and its analogues have shown the most robust ACh-releasing effect in this area of the brain. The analeptic actions of TRH and [Glu2]TRH were measured by the reduction of barbiturate narcosis in mice.

Results: We have shown that [Glu2]TRH has no effect on ACh-release in the rat hippocampus and an attenuation of the TRH-induced ACh-release occurrs upon co-administration [Glu2]TRH with TRH. Changing the stereo-configuration of the central residue in [Glu2]TRH rendered the peptide inactive in the paradigms used in our study. We also have demonstrated that an interplay between TRH and [Glu2]TRH in the brain was also manifested upon a neuropharmacological evaluation using the best-known CNS effect of TRH (its analeptic action) as a paradigm.

Conclusions: [Glu2]TRH attenuates ACh release induced by TRH and TRH's cholinergic antipentobarbital action, which indicates that [Glu2]TRH opposes the effect (i.e., a negative modulator) of TRH in the CNS. [Glu2]TRH may, therefore, play a role in the regulation of the recently proposed neurobiological function of TRH to promote homeostasis.

Sponsor: *NIH*

1200 *(Poster)*

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Classification: GSBS Student

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COMPONENTS OF GERIATRIC DEPRESSION AND TYPES OF COGNITIVE IMPAIRMENT

Purpose: : Purpose: The Geriatric Depression Scale (GDS) is a widely used instrument for assessing depressive symptomatology in the elderly. Previous research on the use of the GDS with cognitively impaired elderly produced a four-factor solution for the 30 item form. These factors were labeled Dysphoria, Meaninglessness, Apathy, and Cognitive Impairment. This study utilized these factors to explore differences in the presentation of depressive symptoms in various types of cognitive impairment.

Methods: : A retrospective chart review was conducted on the records of two hundred twenty eight consecutive cases of community dwelling elderly (Mean Age =78.9 years) who had a neuropsychological evaluation at a metropolitan outpatient clinic and received a diagnosis of Alzheimer's Dementia (AD), Vascular Dementia (VaD) or Mild Cognitive Impairment (MCI). Analyses of differences between the diagnostic groups using discriminate function analyses and ANOVAs were carried out on the four factors and total GDS score.

Results: Results: No differences ($p<.05$) between the groups were found for total GDS score or severity of cognitive impairment. Discriminate function analysis revealed one significant function composed of the apathy, dysphoria and meaninglessness scales accounting for 91.7% of the between group variance (Wilks' Lambda = .902, Chi2 =22.992 (8) p.>.003). VaD patients differed from AD patients scoring significantly higher on the apathy, dysphoria and meaninglessness scales but not higher than the MCI patients. None of the groups differed on the cognitive impairment scale.

Conclusions: Results support the use of the factor scores to distinguish characteristics of affective presentation of different forms of cognitive impairment. Results support the "emotional amnesia" found in Alzheimer's Disease and the relatively greater negative affect found in Vascular Dementias both ischemic and stroke related. The effect of the subjective experience of cognitive changes in MCI may account for the increased level of negative affect

Sponsor: *N/A*

1201 *(Poster)*

Author: Susan Franks

Presenter: Susan Franks

Department: Psychology

Classification: Faculty

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CHANGES IN EATING BEHAVIOR AS A RESULT OF LAPAROSCOPIC BANDING SURGERY

Purpose: The purpose of this study was to examine the effect of laparoscopic banding surgery (LBS) on patterns of eating behavior in morbidly obese patients.

Methods: Subjects included 32 morbidly obese patients (MO) and 14 age-matched normal weight controls (NW). Prior to (T1) and 6-months after LBS (T2), MO were assessed for eating behavior patterns using The Eating Inventory.

Results: At T1, MO and NW significantly differed in Cognitive Restraint (7.6±4.2 vs. 9.8±5.0, p=.0001 respectively), Disinhibition (11.3 ±3.0 vs. 4.1±2.4, p=0.0001), and Hunger (8.4±3.4 vs. 3.8±2.8, p=0.0001). LBS resulted in significant changes in eating behaviors from T1 to T2: Cognitive Restraint (7.6±4.2 vs. 14.2±3.4, p=.0001 respectively), Disinhibition (11.3±3.0 vs. 6.1 ±3.2, p=0.0001), and Hunger (8.4±3.4 vs. 3.7±2.8, p=0.0001).

Conclusions: The pattern of eating behaviors at T2 was not significantly different from NW. Thus, LBS appears to normalize eating patterns and consequently provide patients with an effective mechanism for sustained weight loss.

Sponsor: *N/A*

1300 *(Poster)*

Author: Daniel Clearfield

Presenter: Daniel Clearfield

Department: Osteopathic Research Center

Classification: Dual Degree Student DO/MS

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ATTITUDES, KNOWLEDGE, AND USE OF OSTEOPATHIC MANIPULATIVE TREATMENT BY OSTEOPATHIC SPORTS MEDICINE PHYSICIANS:

Purpose: While all osteopathic physicians are trained in osteopathic manipulative treatment (OMT), some choose not to use it in their practice. Recent surveys have shown a decline in the use of OMT by osteopathic physicians as a whole. There is currently no literature supporting whether osteopathic sports medicine physicians (OSMP) use OMT on a regular basis. This project aims to assess the frequency of OSMPs' use of OMT. For OSMPs who do not use OMT, this survey will discern the reasons why they do not.

Methods: This study was a cross-sectional survey. 2 surveys and 1 reminder postcard were mailed to OSMPs over a 10-week period. The mailing lists were provided by the American Osteopathic Association and the American Osteopathic Academy of Sports Medicine. The survey was a 33-item self-reported instrument designed to be completed in 5 minutes. 23 of the items were scored on a Likert scale, and 10 items recorded OSMP demographic and medical practice information. Using SPSS, basic descriptive statistics were computed for socio-demographic characteristics, training, practice characteristics, and responses to the attitudes, knowledge and use survey items. Inferential statistical analyses were used to explore the relationships among survey variables. Statistical significance was assessed at the .05 level.

Results: A total of 252 OSMPs were eligible to participate in the study and 196 surveys (77.8% response rate) were returned. There was a significant association between post-graduate training and frequency of using OMT in sports medicine practices (p=.01). When directly comparing AOA and ACGME post-graduate training, AOA-trained OSMPs were shown to practice significantly more OMT than their ACGME counterparts (p=.04). There was not a statistically significant association between fellowship training and frequency of OMT use in sports medicine practices (p=.25). Similarly, there were no significant differences in frequency of OMT use in sports medicine practices when graduates of AOA and ACGME fellowships were directly compared (p=.30).

Conclusions: The results of this study demonstrated a large number of OSMPs (41% of respondents) use OMT in their sports medicine practice <20% of the time. It was shown there is a statistically significant number of AOA-trained OSMPs that practiced more OMT than their ACGME and dually-accredited counterparts. The results of this study may have an impact upon residency selection for students wishing to pursue a career in sports medicine with an OMT emphasis.

Sponsor: *Osteopathic Research Center*

1301 *(Poster)*

Author: John Licciardone

Presenter: John Licciardone

Department: Osteopathic Research Center

Classification: Faculty

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A PILOT CLINICAL TRIAL OF OSTEOPATHIC MANIPULATIVE TREATMENT IN PREGNANCY

Purpose: The primary purpose of this pilot clinical trial was to explore the potential therapeutic effects of OMT during the third trimester of pregnancy.

Methods: This study was conducted by the Osteopathic Research Center at the University of North Texas Health Science Center at Fort Worth (UNTHSC). All study procedures were approved by the Institutional Review Board and the study was registered with ClinicalTrials.gov (NCT00298935). Exclusion criteria included either of the following: (1) intent to deliver at a non-participating hospital or (2) high risk pregnancy as determined by the attending obstetrician. Each subject was randomized to one of three treatment groups: (1) usual obstetrical care and OMT (UOBC+OMT); (2) usual obstetrical care and sham ultrasound treatment (UOBC+SUT); and (3) usual obstetrical care only (UOBC only). The UOBC+OMT and UOBC+SUT groups were scheduled to receive treatments at the 30th week (visit 1), 32nd week (visit 2), 34th week (visit 3), 36th week (visit 4), 37th week (visit 5), 38th week (visit 6), and 39th week (visit 7). The primary outcome measures included: (1) an 11-point scale for the typical or average level of back pain; (2) the Roland-Morris Disability Questionnaire (RMDQ); and (3) the SF-12 Version 2 Health Survey (SF-12) scale score for bodily pain and the summary scores for physical health and mental health. All analyses were based on the intention-to-treat principle. Missing data for all primary outcomes were imputed using the "carry-forward" method.

Results: A total of 49, 48, and 49 subjects were randomized to the UOBC+OMT, UOBC+SUT, and UOBC only groups, respectively. Typical or average back pain levels decreased over time in the UOBC+OMT group, remained essentially unchanged in the UOBC+SUT group, and increased in the UOBC only group (ANCOVA treatment group x time interaction, P=.02). The Roland-Morris disability scores increased over time in all three treatment groups; however, the rate of increase was significantly different among the groups (P<.001). Disability progressed less rapidly in the UOBC+OMT group than in the UOBC only group (P<.001), and there was also a trend toward less rapid progression in the UOBC+OMT group in comparison with the UOBC+SUT group (P=.08).

Conclusions: OMT was associated with favorable disability and pain outcomes during the third trimester of pregnancy.

Sponsor: *Osteopathic Heritage Foundation*

1302 *(Poster)*

Author: John Licciardone

Presenter: John Licciardone

Department: Osteopathic Research Center

Classification: Faculty

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SYSTEMATIC REVIEW AND META-ANALYSIS: OSTEOPATHIC MANIPULATIVE TREATMENT

Purpose: The purpose of this project is to conduct a systematic review of the literature on osteopathic manipulative treatment (OMT). The three primary objectives are: (1) to provide evidence on the cost effectiveness of OMT; (2) to update a previous meta-analysis of OMT for low back pain; and (3) to perform meta-analyses, if feasible, for OMT of other conditions.

Methods: A bibliographic search of the osteopathic literature was conducted beginning in May 2006. This was followed by footnote chasing and consultation with other osteopathic investigators as needed. Identified citations, including available abstracts, were then reviewed by three independent and blinded reviewers. The full-length reports were retrieved for review if any of the three reviewers selected the relevant citation for further evaluation based on the likely presence of research involving OMT in human subjects, conducted by osteopathic investigators. The retrieved full-length reports were then evaluated in greater detail for the presence of research involving OMT in human subjects, conducted by osteopathic investigators. The reports having the latter characteristics were coded for study design elements, focus of study, target population, country where study was conducted, medical discipline, organ system studied, and study scale.

Results: Through December 2006, 2189 citations had been screened for relevance to OMT, and 963 (44%) were selected for evaluation of the corresponding full-length manuscripts. Of these 963 manuscripts, 204 (21%) were selected for more detailed evaluation. With regard to the first project objective, of these 204 manuscripts, 22 (11%) involved cost effectiveness and 15 (7%) included useful data for the potential development of recommendations on the cost effectiveness of OMT. Findings of the cost effectiveness review are currently embargoed pending a final report to the sponsoring agency.

Conclusions: There is a sizeable and diverse base of osteopathic literature available for systematic review. Final results for all three project objectives are anticipated later this year.

Sponsor: *American Osteopathic Association*

1303 *(Poster)*

Author: Lisa Hodge

Presenter: Lisa Hodge

Department: Osteopathic Research Center

Classification: Faculty

Lisa M. Hodge 1,3, Hollis H. King 3, Arthur G. Williams, Jr. 2, Stephanie J. Reder 1 , Tejaswi Belavadi 1, Jerry W. Simecka 1, Scott T. Stoll 3, H. Fred Downey 2. Department of Molecular Biology and Immunology 1, Department of Integrative Physiology 2, Osteopathic Research Center 3, The University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107.

ABDOMINAL LYMPHATIC PUMP TREATMENT INCREASES LEUKOCYTE COUNT AND FLUX IN THORACIC DUCT LYMPH

Purpose: Studies suggest that lymphatic pump techniques (LPT) enhance immunity and resistance to infection, but direct evidence of this has not been documented. In this study, the immediate effects of LPT on lymph flow and leukocyte flux in the canine thoracic lymph duct were measured.

Methods: Lymph flow was measured by timed collection, and lymph was collected at baseline conditions and during LPT.

Results: The baseline leukocyte count was 4.8 ± 1.7 million cells/ml, and LPT increased leukocytes to 11.8 ± 3.6 million cells/ml ($P < 0.05$). Whereas numbers of macrophages, neutrophils, total lymphocytes, T cells and B cells increased similarly during LPT, their relative percentage in lymph was unaltered by LPT; however, IgA antibody forming B cells increased from 5.8% at baseline to 17% during LPT. These data suggest that LPT acts preferentially on mucosal tissues, a source of these mobilized leukocytes. Furthermore, LPT enhanced lymph flow 4-fold. Leukocyte flux was computed from the product of lymph flow and cell count. LPT enhanced leukocyte flux from 8.2 ± 4.1 million total cells per min to 60 ± 25 million cells per min.

Conclusions: In summary, LPT significantly increased both thoracic duct lymph flow and leukocyte count, so lymphatic leukocyte flux was markedly enhanced. Enhanced mobilization and lymphatic transport of immune cells during abdominal compression is likely an important mechanism responsible for the increased immune responses of patients treated with LPT.

Sponsor: *NIH Grant U19 AT2023-01*

1304 *(Poster)*

Author: Dennis Minotti II

Presenter: Dennis Minotti II

Department: Osteopathic Manipulative Medicine

Classification: Postdoctoral Fellow/Resident

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A CASE STUDY OF MERALGIA PARESTHETICA: AN OSTEOPATHIC APPROACH

Purpose: Meralgia paresthetica (MP) is a mononeuritis of the lateral femoral cutaneous nerve (LCN). MP results from an entrapment of the LCN anywhere along its anatomical course, which begins at the L2-L3 nerve roots and ends in the anterior-lateral thigh. The most common site of entrapment occurs where it exits the pelvis, medial to the anterior superior iliac spine. The resulting compression of this sensory nerve can lead to the following symptoms: pain, paresthesias, and/or dysthesias of the anterior-lateral thigh. This anatomical compression, which can be significantly influenced by somatic dysfunction, makes Osteopathic Manipulative Treatment (OMT) a valuable addition to conservative management of MP.

Methods: The patient is a 57-year-old police officer with a history of several months of dull burning pain and numbness in his left anterior-lateral thigh that progressively worsened and became almost constant. The pain/numbness was exacerbated by prolonged sitting while wearing a gun belt and by walking long distances. Physical examination showed decreased sensation to light touch over the LCN distribution of his left leg with normal strength (+5/5) and reflexes (DTRs 2/4) bilaterally. The patient was also found to have significant somatic dysfunction upon examination. These dysfunctions included L1-4 NSLRR, left iliopsoas tender points and restriction, a left posterior innominate, and restriction of the left lower extremity to extension at the hip joint.

Results: The patient was treated 4 times over a 3-month period with OMT focusing on the regions of somatic dysfunction previously mentioned. After the fourth treatment, the patient reported near complete resolution of the burning pain and only intermittent numbness in the left thigh.

Conclusions: Since meralgia paresthetica is most commonly a result of anatomic compression, it is reasonable to conclude that decompressing the LCN by correcting somatic dysfunction can significantly reduce a need for invasive procedures. In addition, OMT, used as a conservative treatment, can accelerate healing of the LCN. MP is an example of how Osteopathic Physicians can utilize our unique knowledge and skills to improve patient care. Based on the significant results of this case, as well as similar cases reported in the literature, a need for further research on the use of OMT in decompression of the LCN exists.

Sponsor: *N/A*

1305 *(Poster)*

Author: Turner Slicho

Presenter: Turner Slicho

Department: Osteopathic Manipulative Medicine

Classification: Postdoctoral Fellow/Resident

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POSTURAL CONSIDERATIONS IN CHRONIC PAIN: A CASE REPORT

Purpose: The purpose of this case report is to examine the effects of postural optimization in a patient with chronic idiopathic musculoskeletal pain which has remained unrelieved by conventional treatment modalities.

Methods: Postural levelness was achieved using: foot orthotics to optimize the shape and alignment of the feet and ankles, heel lift to level the sacral base measured radiographically, and osteopathic manipulation to reduce tightness and tenderness of tissue.

Results: Over the course of treatment, the patient reported a significant decrease in chronic musculoskeletal pain. Post-treatment radiography shows a decrease in scoliotic curvature of the spine, as well as resolution of sacral base unleveling.

Conclusions: While anecdotal evidence is promising, further investigation into the efficacy of postural optimization in the relief of chronic musculoskeletal pain is necessary.

Sponsor: *N/A*

1400 *(Poster)*

Author: Walter McConathy

Presenter: Sulabha Paranjape

Department: Internal Medicine

Classification: Faculty

Walter McConathy, Sulabha Paranjape, Maya Nair, Stanley Stevens, Jr.*, Linda Mooberry*, Laszlo Prokai*, and Andras Lacko*. Department of Medicine, TCOM, and *Department of Molecular Biology & Immunology, Graduate School of Biomedical Sciences, University of North Texas Health Sciences Center*

LIMITED PROTEOLYTIC DEGRADATION OF THE MAJOR APOLIPOPROTEIN OF HDL, APOA-I, ALTERS LIPID BINDING PROPERTIES OF TRUNCATED APOA-I.

Purpose: In our studies developing reconstituted high density lipoproteins (rHDL) as a vehicle to deliver hydrophobic chemotherapeutic agents, we noted that apolipoprotein A-I (apoA-I) can undergo limited proteolysis in several different preparations. The objective of the present set of studies was to better define this proteolysis and thereby control it.

Methods: A limited proteolysis was first noted using a novel affinity chromatography step to isolate apoA-I from plasma in large quantities. In some of these preparations apoA-I was partially degraded. Precautions, e.g., proteolytic inhibitors were somewhat effective in preventing this. We also found that preparations of apoA-IZ from an industrial source (ZLB), had the same pattern for apoA-I if these preparations were in solution for extended periods of time at 6oC.

Results: Examining several different preparations of apoA-IZ, we could demonstrate 2 major forms of apoA-I, intact (apoA-Ii) and truncated (apoA-IT), both by staining of 14% SDS PAGE gels and immunoblots with anti-apoA-I. Incubation of apoA-Ii at 37oC, demonstrated limited proteolysis: only apoA-IT was present at MW = 26,600 KDal indicating a loss of a polypeptide(s) of 2,300 KDal. These findings were verified by mass spectrometry with sequence analysis underway to identify the polypeptide(s) of interest. Ultracentrifugation of rHDL prepared with intact apoA-Ii and a mixture (apoA-Ii + T) gave different patterns of apoA-I's distribution, the intact form, apoA-Ii, resided primarily in the HDL fraction while the apoA-IT was found almost exclusively in the lipid poor fraction (VHDL).

Conclusions: Further studies are ongoing to better define and control this processing. These studies demonstrate that apoA-I isolated by procedures other than ultracentrifugation contains a protease that degrades apoA-I in a stepwise process. The apoA-IT though only slightly shorter than its parent, has lost the ability to form rHDL and therefore has lost considerable lipid binding properties. Literature reports loss of lipid binding of recombinant apoA-I if it is truncated at both the C- and N-terminal. This apoA-IT offers the possibility to probe the functional properties of apoA-IT contrasted with apoA-Ii. Intriguing additional questions to be addressed are whether this protease processing is physiological, which protease is involved, and is the processing of apoAI related to HDL-cholesterol levels or other physiological functions of apoA-I.

Sponsor: *N/A*

1401 *(Poster)*

Author: Pabak Sarkar

Presenter: Pabak Sarkar

Department: Molecular Biology and Immunology

Classification: GSBS Student

Pabak Sarkar, Shashank Bharill, Ignacy Gryczynski, Zygmunt Gryczynski, Maya P. Nair and Andreas G. Lacko UNTHSC, Fort Worth, TX 76107

BINDING OF 8-ANILINO-1-NAPHTHALENESULFONATE TO LECITHIN: CHOLESTEROL ACYLTRANSFERASE STUDIED BY FLUORESCENCE TECHNIQUES

Purpose: Lecithin: cholesterol acyltransferase (LCAT) is an important enzyme involved in reverse cholesterol transport. Crystal structure of this 62 kDa glycoprotein is not available and there is limited information about its structure. The purpose of this study is to gain insight about the structure of LCAT using steady-state and time-resolved fluorescence spectroscopy with solvatochromic fluorophore 8-Anilino-1- naphthlenesulfonate (ANS) and characterize conformational dynamics of LCAT-ANS complex using fluorescence anisotropy.

Methods: We observed the steady-state fluorescence of LCAT in absence and presence of ANS using excitation at 295 nm for excitation of tryptophan (trp) residues. Then, we measured changes in fluorescence lifetimes of trp residues in LCAT (using excitation wavelength of 295 nm and emission wavelength of 340 nm) with progressive addition of ANS to study tryptophan quenching due to LCAT-bound ANS. We also measured fluorescence spectrum and lifetimes of ANS (bound to LCAT) with excitation wavelength of 375 nm which exclusively excites ANS. For time resolved studies, observation was at 480 nm; characteristic to ANS fluorescence. Both steady-state and time-resolved anisotropies were studied for the LCAT-ANS complex using 375 nm excitation wavelength.

Results: Fluorescence spectra and intensity decays show an efficient energy transfer process from trp residues to ANS. Using FRET model, the apparent distance from trp donor to ANS acceptor was calculated to be 2.74 nm from the changes in donor lifetime. The ANS to LCAT binding constants were estimated from two independent titrations, a) ANS with constant concentration of LCAT, and b) LCAT with a constant concentration of ANS. The data were fitted to the kinetic model and binding constants were calculated to be 1.73 and 3.26 in the first case. The ANS labeled LCAT fluorescence anisotropy decay revealed the correlation time of 42 ns with a weak residual motion of 2.8 ns characteristic for macromolecule of about 60 kDa molecular mass.

Conclusions: The fluorophore ANS, not fluorescent in the polar solvents, binds to more than one hydrophobic sites of LCAT and display useful fluorescence emission. It quenches the trp fluorescence due to FRET mechanism. The fluorescence of ANS-labeled LCAT protein can be used to study a rotational mobility of the macromolecule. Using anisotropy measurements the interaction of LCAT with other large macromolecules can be studied with.

Sponsor: *N/A*

1402 *(Poster)*

Author: Navin Rauniyar **Presenter:** Navin Rauniyar

Department: Molecular Biology and Immunology **Classification:** GSBS Student

Navin Rauniyar (1), Katalin Prokai-Tatrai (2), Stanley M. Stevens (1), Laszlo Prokai (1). Department of Molecular Biology and Immunology (1), Department of Pharmacology & Neuroscience (2). The University of North Texas Health Science Center, 3500 Camp Bowie Blvd, Fort Worth, TX 76107.

MASS SPECTROMETRIC CHARACTERIZATION OF CARBONYLATED PEPTIDES

Purpose: The main objective of this study is to implement a mass spectrometric-based approach for identification of the structure and sites of modification by reactive carbonyl compounds using the model peptides, a 13 amino acids long myelin basic protein (MBP) fragment and oxidized insulin B chain from bovine pancreas, respectively.

Methods: MBP fragment (87-99) from Bachem and oxidized insulin B chain from Sigma (131 μM) were reacted separately with HNE (2 mM). In parallel, MDA modification was performed by incubating MBP fragment (131μM) with MDA (2mM). After removal of unreacted substances and desalting by solid phase extraction, the samples were analyzed by LC-ESI MS. Tandem mass spectrometric (MS/MS) analyses were carried out on both peptides to obtain information regarding site of modification.

Results: Our study suggests that carbonylation of peptides occurs readily and is highly selective for particular amino acid residues. We found that HNE forms a Michael adduct with the imidazole ring of histidine, increasing the peptide mass by 156 Da/adduct in MBP and oxidized insulin B chain. The same compound also undergoes Schiff base addition with the e-amino group of lysine which results in a 138 Da increase in mass of the modified peptide. MDA, however, predominantly forms Schiff base adducts with the e-amino group of lysine. MS/MS spectra of the doubly charged ions at m/z 792.44, 834.9 and 843.49 of MBP fragment showed that MDA formed a Schiff base adduct with lysine whereas HNE underwent Schiff base addition with lysine or formed a Michael adduct with histidine. In case of oxidized insulin B chain MS/MS spectra of triply charged ions at m/z 1218.26 and 1270.30 showed either one or both histidines were modified by HNE. Also, traces of Schiff base additions of HNE to lysine residues were observed.

Conclusions: Using mass spectrometry, it was possible to identify that histidines were the primary target residues for covalent modification by HNE, via Michael-addition rather than lysine that undergoes Schiff base addition. This study will facilitate our understanding of the reactivity and selectivity of covalent modifications of protein by the end products of lipid peroxidation during oxidative stress. This in turn will help in elucidating mechanisms for prevention or provide a conceptual framework for the future design of drug treatments. (Research supported by the NIH grant AG025384)

Sponsor: *NIH*

1403 *(Poster)*

Author: Jingwei Fu **Presenter:** Jingwei Fu

Department: Molecular Biology and Immunology **Classification:** GSBS Student

Jingwei Fu, Stanley M. Stevens, Jr., Laszlo Prokai Department of Molecular Biology and Immunology University of North Texas HSC, Fort Worth, Texas

PROTEIN EXPRESSION PROFILING OF MAMMALIAN SYNAPSES BY SUBCELLULAR FRACTIONATION AND MASS SPECTROMETRY-BASED PROTEOMICS

Purpose: Exploration of the brain by the methods of proteomics has apparent advantages. Identification of the presence of proteins in key compartments of neurons and glia will provide an essential framework for understanding their functions. Protein expression analysis allows us to identify proteins actively involved in the progression of diseases and to develop strategies for molecular intervention by knowledge of the biological pathways associated with diseases. The effects of drugs can also be thoroughly characterized and compared to allow for a detailed interrogation of their mechanism of action.

Methods: Analysis of protein constituents in organelles and specifically isolated subcellular fractions or protein complexes appears to be a viable subproteome approach that reduces complexity and allows for a meaningful interpretation of protein data for brain research. Methods to obtain synaptic junctions by sucrose gradient and further fractionation to presynaptic active zone and postsynaptic density (PSD) fractions by detergents and stepwise pH adjustment to release proteins from the synaptic junction "scaffold" have been used to focus studies our target subproteome. SDS-PAGE was used as the first dimension of additional protein separation followed by in-gel trypsin digestion and LC/ESI-MS and MS/MS analysis of the tryptic peptides. Protein identifications were done by database search.

Results: Protein expression profiling for the synaptic membrane protein (SMP) fraction and two of its subfractions has been done. Compared to earlier reports, our study has been the fist that completed protein expression profiling of mammalian synapses with high confidence by using high resolution and high mass accuracy mass spectrometry.

Conclusions: The PSD fraction was rich in receptor and transporter proteins, which validated our experimental strategy. Further studies hold the promise of understanding of synaptic protein networks and potential identification of potential disease biomarkers.

Sponsor: *N/A*

1500 *(Poster)*

Author: Alberto Coustasse

Presenter: Alberto Coustasse

Department: Health Management and Policy

Classification: Faculty

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DISPARITIES AMONG HISPANIC SUBGROUPS: SELF-REPORTED ADL AND IADL DISABILITY RESULTS FROM THE NATIONAL HEALTH INTERVIEW SURVEY 2001-2003

Purpose: The purpose of this study was to compare rates of self-reported disability and functional limitation among Hispanic subgroups using data from older adults sampled in the 2001-2003 National Health Interview Survey (NHIS).

Methods: The study sample included 31,875 individuals aged 65 years and above who were randomly selected from the (NHIS) from 2001 to 2003. The two dependent variables were: disabilities from ADL (Activities of Daily Living) and IADL (Instrumental Activities of Daily Living).The independent variables included four major groups: Non-Hispanic whites, Non-Hispanic Blacks, Hispanics and others. Hispanics were further classified into five major subgroups: Puerto Ricans, Mexican/Mexican Americans, Cuban/Cuban Americans, Central and South (C&S) Americans and Other Hispanic. Also were used gender, age, educational attainment, and annual household income.Chi-square analysis was applied for bivariate comparisons and multiple logistic regression analysis was used to estimate Odds Ratios to determine risk for models predicting ADL and IADL disability by ethnicity and Hispanic subgroups.

Results: Results revealed a 19.5% disability of any type. Within Hispanic subgroups, Puerto Ricans reported the highest rates of ADL, IADL or any disability (35.4%) compared with other Hispanic subgroups and higher than non Hispanic blacks (28.2%). Cubans showed the lowest rate in any disability (15.9%) within Hispanics and even lower than non Hispanic whites (17.7%). From our logistic regression models, for both ADL and IADL disability, respondents who were older, female, non Hispanic blacks or Hispanics showed significantly higher rates than their white counterparts ($p<0.01$).Within Hispanics subgroups, only Mexicans and Mexican Americans males were significantly less likely to report ADL disability than females ($p<0.01$)and IADL, but did not reach statistical significance ($p>0.05$).

Conclusions: The findings highlights the high rate of inter group variability among the US population.The most severe health problems in the United States are concentrated among minority groups; and older adults from disadvantaged backgrounds bear a substantial burden. The fact is since disabilities increase with age and our population is living longer,the number of individuals with activity, work or functional limitations will increase and will constitute a real public health problem where there is too much knowledge about it and few concrete solutions.

Sponsor: *N/A*

1501 *(Oral)*

Author: Theresa Quiroz

Presenter: Theresa Quiroz

Department: Social and Behavioral Sciences

Classification: SPH Student

Theresa D. Quiroz, Alberto Coustasse, Sue Lurie School of Public Health, University of North Texas Health Science Center at Fort Worth, Texas, 76107

TO THE BITTER END: DISPARITY ISSUES IN END OF LIFE CARE IN THE UNITED STATES

Purpose: The purpose of this study was to analyze historical, clinical, cultural and ethical issues in the End Of Life (EOL) care, with a special emphasis on disparities in minority patient care, using hypothetical case studies retrieved from referenced literature.

Methods: To compare many perspectives on EOL care disparities, a total of three hypothetical cases extracted from the literature were presented, each case with its own health care issue related to decisions for palliative care. These cases represent three different racial or ethnic groups, and two genders. The basis of comparison was to evaluate the literature on barriers to quality for EOL care for each group.

Results: The review of literature included comparison of national standards of EOL care that were developed by consensus among health care organizations, professions, patients, and families. These standards are presented, followed by the three cases in EOL care extracted from the literature. Cases represented patient diversity in gender, race/ethnicity, cultural traditions, religious/spiritual beliefs and health issues for elderly patients. Analysis of ethical issues found treatment or palliative care decisions varied among patients, families and clinicians. For the Hispanic woman, family support, communication and religious beliefs affected decisions. For the African-American man, perception of discrimination was an issue. For the Asian-American man, cultural beliefs, family roles and expectations challenged medical decisions. Interpretations of ethical principles - autonomy, beneficence, non-malfeasance and justice - varied among cases.

Conclusions: Based on the above cases, it is evident that language and cultural understanding are essential for providing compassionate quality care to ethnically and racially diverse patients. In their absence, such barriers would likely hinder access to much needed information and support for patients and families. There is potential for improving the delivery of palliative care, to be more available to patients and sensitive to psychosocial needs. Those patients and families who have been marginalized and discriminated against are the only ones who can teach the world how to make those improvements.

Sponsor: *N/A*

1502 *(Poster)*

Author: Jotam Pasipanodya
Presenter: Mauricio Vecino
Department: School of Public Health
Classification: SPH Student

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USE OF ST GEORGE'S RESPIRATORY QUESTIONNAIRE IN TREATED TUBERCULOSIS

Purpose: Pulmonary tuberculosis results in pulmonary function loss despite microbiologic cure in almost 60% of patients. There are no recommendations for evaluation or other follow-up on completion of tuberculosis therapy. Health related quality of life (HRQoL) questionnaires allow clinicians to measure disease impact on a person's daily life. These may be useful to identify persons with and without post tuberculosis pulmonary impairment. There is no disease-specific instrument validated for persons with tuberculosis.

Methods: We completed the St. George's Respiratory Questionnaire (SGRQ), an instrument validated for several types of lung disease, with patients diagnosed with culture-positive pulmonary tuberculosis or with latent tuberculosis infection (LTBI). SGRQ results were correlated with pulmonary function tests. Stability and validity of the SGRQ was tested. Outcomes measured were health-related quality of life pattern changes attributable to pulmonary tuberculosis and the usefulness of the SGRQ in their ascertainment.

Results: The SGRQ total score is stable and significantly correlates with pulmonary function and a previously validated long form health related quality of life questionnaire. Scores correlated with pulmonary function for all participants regardless of tuberculosis history (Pearson correlation range = 12 - 25%). Scores increased as predicted pulmonary function decreased. Mean total score for subjects with PTB was 13.2 units higher than controls (score (SE) 23.5(2.2) vs. 10.3(1.0), respectively, $p<0.001$). Sensitivity of the SGRQ total score in predicting pulmonary function ranged from 90% to 14.5%, with greater sensitivity for patients with more pulmonary function. The specificity and positive predictive value (PPV) of total scores for pulmonary function ranges from 16 to 91% and PPV of 60.2 to 69.2%.

Conclusions: There is a health difference in those who have had TB and those who have not, and the SGRQ is a valid means with which to measure it. Cure of pulmonary tuberculosis marks the onset of post-tuberculosis pulmonary impairment in almost 60% of patients. We found the SGRQ gives valid measure of health related quality of life in patients with history of pulmonary tuberculosis. We found a mean 13% health loss attributable to PTB despite clinical cure. We found a significant difference in health between our control group with LTBI and population normals, suggesting HRQoL instruments may be sensitive to health loss not easily identifiable by means such as spirometry. Measurement of HRQoL lost to pulmonary tuberculosis allows more accurate estimation of population health that can be gained through tuberculosis prevention

Sponsor: *N/A*

1503 *(Poster)*

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Presenter: Jotam Pasipanodya
Department: School of Public Health
Classification: SPH Student

Jotam Pasipanodya

POST PULMONARY TUBERCULOSIS IMPAIRMENT SCREENING –A RATIONALE FOR INFLUENZA VACCINATION

Purpose: There is no consensus regarding the management of post tuberculosis pulmonary impairment (PTPI). We determined the cost-effectiveness of screening for post pulmonary tuberculosis impairment (PTPI) and evaluated the effectiveness of annual influenza vaccination of high-risk patients. Standard practice of no screening and no vaccinations were the comparator.

Methods: We calculated the incremental cost-effectiveness of screening and vaccinations interventions in subjects who had completed treatment for tuberculosis using a decision tree and a hypothetical cohort of 100 individuals. Net health benefits (disease events, physician visits, hospitalizations, long term sequelae and deaths averted), incremental dollar cost (2003) quality adjusted life years (QALYs) gained were evaluated. All costs (direct medical costs and patients' times) were included and benefits were discounted at three per cent per year

Results: Screening pulmonary tuberculosis patients for impairment after treatment and vaccinating at risk patients for influenza is cost-effective and provides net health benefits compared to non-screening. Annual inactivated influenza vaccination of low and high-risk patients would reduce influenza episodes by 33 and 36 percent per year and avert 3 and 6 deaths per 100000 respectively, while live attenuated influenza vaccination would also reduce influenza episodes by 40 and 44 percent and avert 2 and 6 deaths per 100000. The incremental cost-effective ratio for inactivated influenza vaccine (IIV) was $28737.67, and $49009.99 for high risk and all patients per Qaly saved, respectively, compared to no vaccination and was still cost effective when both higher and lower thresholds of vaccine effectiveness in preventing influenza illness were considered Cost effectiveness ratios for live attenuated influenza vaccine were higher.

Conclusions: Screening patients with a history of pulmonary tuberculosis for pulmonary impairment combined with targeted influenza vaccination has net health benefits and is a cost-effective intervention. HIV co-infection increases the benefit of the intervention in countries with high prevalence that can afford to pay for the intervention. More research is required to fully understand the full cost-effectiveness of post-tuberculosis particularly in countries with high prevalence both TB and HIV but low resources.

Sponsor: *N/A*

1504 *(Poster)*

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RACIAL DISPARITIES IN PATIENT-PHYSICIAN RACIAL CONCORDANCE

Purpose: Patient-physician racial concordance has been associated with positive health outcomes including the use of preventive services and satisfaction with care. Patient-physician race concordance may contribute to high-quality patient-provider encounters by removing barriers to open communication and establishment of rapport and trust with providers. The objective of this study is to investigate racial disparities in patient-physician racial concordance.

Methods: This cross-sectional study examines data from the 2001 Commonwealth Fund Health Care Quality Survey. The study population was a nationally-representative sample of 6,306 adults age 18 and older. Racial disparities in patient-physician racial concordance were examined using bivariate and multivariate analyses. Logistic regression analysis was used to identify correlates of patient-physician racial concordance. Three race-specific (Non-Hispanic White, Black or Hispanic) multivariate logistic regression analyses were performed to identify disparities in correlates of patient-physician racial concordance.

Results: In both bivariate and multivariate analyses, racial disparities in patient-physician racial concordance were observed, with the highest rates of concordance for whites (65.5%), followed by Blacks (21.2%), Hispanics (20.2%), and Asian and Pacific Islanders (3.1%). Other characteristics associated with racial concordance were older age (OR 1.17, 95% C.I. 1.12-1.21), female gender (OR 1.36, 95% C.I. 1.22-1.53), being married (OR 1.31, 95% C.I.1.17-1.46), higher educational attainment (OR 1.12, 95% C.I. 1.07-1.18), having insurance (OR 1.91, 95% C.I. 1.49-2.46), and having been born in the United States (OR 4.56, 95% C.I. 3.91-5.33).

Conclusions: Patient-physician race concordance occurs at much lower rates for racial and ethnic minorities. Furthermore, our findings suggest that patient-physician race concordance is associated with other established predictors of improved health outcomes (e.g. higher socioeconomic status, being married, having insurance). While factors such as socioeconomic status are difficult to change, patient-physician race concordance is a factor over which individual patients can exercise control. Employing strategies to create concordant patient-physician dyads may be a plausible mechanism by which health disparities can be reduced. Policies to enhance recruitment of minority physicians are important in growing the number of concordant physicians available to growing minority populations.

Sponsor: *N/A*

1505 *(Poster)*

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THE EFFECTS OF A MENTAL HEALTH EDUCATION PROGRAM ON KNOWLEDGE AND ATTITUDES OF MENTAL HEALTH IN EIGHTH GRADE STUDENTS

Purpose: Traditionally, mental health in the schools has been limited to providing clinical services, and education has been minimal. Rarely has mental health information been disseminated into middle schools. Understanding what constitutes a healthy mental life, problems common to adolescents, and reducing stigma are components of this education.

Methods: The project was implemented for eighth grade students in urban and suburban schools in Texas during Spring 2006. A chi-square analysis was performed on pre/post self-report questions related to general mental health, etiology/treatment of mental illness, quality of life of the mentally ill, and stigma.

Results: Results showed that perceived knowledge about mental illness improved after the program. Knowledge about stigma and mental illness was also high including beliefs that people with a mental illness can lead a normal life, people with a mental illness can be discriminated against, and mental illness can inflict anyone. However, there was not a significant change in attitudes related to stigma. It seems that these students did not change their feelings regarding the ability of mentally ill people to live in the community without adversely affecting it.

Conclusions: This analysis revealed that although knowledge can be readily improved, a change in attitudes could not occur from just one iteration of the educational program. However, mental health education is still needed in middle schools and educational programs may require multiple exposures and further evaluation.

Sponsor: *SAMHSA*

1506 *(Oral)*

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RACIAL/ETHNIC HEALTH DISPARITIES IN UNITED STATES POULTRY WORKERS

Purpose: The main objective of this proposed retrospective cohort study is to investigate racial/ethnic health disparities between poultry slaughtering/processing plant workers who belonged to the United Food & Commercial Workers International Union from 1949-2003.

Methods: Several union records were used to identify 30,000 subjects who worked in poultry plants where high exposures to poultry oncogenic viruses occurred. Data collection included requesting death certificates of the subjects identified. Statistical analyses of cancer mortality risk will be conducted by estimation of standardized mortality, standardized proportional mortality ratios and relative risks. Cancers occurring in excess that exhibit racial disparities will be examined in greater detail to identify whether any observed racial and ethnic disparities for a given cancer type(s) can be explained by occupational factors, non-occupational factors or both. The subject's next of kin will be asked to participate in the study by answering questions pertaining to the deceased's medical, lifestyle and occupational history.

Results: The anticipated results of this research will assess racial/ethnic disparities that are well-known to exist in the general population to determine if they are also observable in the lowest socioeconomic group and within an occupational setting, by examining cancer mortality in poultry workers.

Conclusions: It is anticipated that identification of risk factors for racial/ethnic disparities in this socially homogeneous group will provide an understanding into the mechanism of racial/ethnic heath disparities.

Sponsor: *N/A*

1507 *(Poster)*

Author: Roberto Cardarellli
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THE MODIFYING EFFECTS OF EDUCATION AND INCOME ON HISPANICS REPORTING PERCEIVED DISCRIMINATION

Purpose: Research shows experiences of discrimination negatively impact health. Little is known regarding whether socioeconomic position modifies reporting of perceived discrimination. This cross-sectional study of sixty-nine participants investigated modifying effects of education and income on reporting of perceived discrimination among Hispanics and whites.

Methods: Subjects participated in the Cardiovascular Disease, Perceived Discrimination, Social Support, and Sense of Control: Understanding Biological Pathways study. Study exclusion included history of cardiovascular disease, less than a year of life expectancy, or pregnancy. Perceived discrimination was measured using Experience of Discrimination (EOD) instrument in English or Spanish. Discrimination was modeled as a dichotomous variable (any experience or no experience of discrimination) and functioned as the outcome variable of interest. Race/ ethnicity was self-reported and categorized as non-Hispanic white, non-Hispanic African American, Hispanic, other. Educational attainment was dichotomized high school degree or less and greater than a high school degree. Income was dichotomized as annual household income of < or = $30,000. Logistic regression was used to estimate odds ratios and 95% confidence intervals for the association between experiences of perceived discrimination and race/ethnicity. To assess effect modification, association between experience of perceived discrimination and race/ethnicity was stratified by education and income, independently.

Results: The logistic regression analyses found Hispanics 3.64 times (95% confidence interval [CI] 1.21-11.00) more likely to report perceived discrimination compared to whites. When stratified by education, Hispanics with a high school education or less were 1.71 (95% CI 0.27-10.92) times more likely to report perceived discrimination compared to whites. This association increased to 4.09 (95% CI 1.31-12.72) among Hispanics with more than a high school education. Similarly, Hispanics with annual household incomes of $30,000 or greater were 4.43 times (95% CI 1.41-13.93) more likely to report perceived discrimination than whites. Those with an income of less than $30,000 were 1.71 times (95% CI 1.13-12.90) more likely to report perceived discrimination than whites.

Conclusions: Reports of perceived discrimination among Hispanics differ by socioeconomic factors (education and income). These results may provide insight to methodological design factors in future studies.

Sponsor: *N/A*

1508 *(Poster)*

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THE RELATIONSHIP BETWEEN PREGNANCY INTENDEDNESS AND HAPPINESS TO BIRTH OUTCOMES OF WOMEN IN THE UNITED STATES

Purpose: The purpose of this study is to understand how pregnancy intendedness and level of pregnancy happiness impact birth outcomes of women in the United States.

Methods: This study utilized the National Survey of Family Growth Cycle 6, administered by the National Center for Health Statistics (NCHS). The sample for this study included White, Hispanic and African American women who were 20 years of age and older. The dependent variable for this study was preterm birth. The two independent variables of interest were pregnancy intendedness and pregnancy happiness. Intended pregnancies are those that occurred at the right time or later than wanted. Unintended pregnancies are those that are mistimed or unwanted pregnancies. Happiness of being pregnant ranged from 1 being very unhappy to 10 being very happy

Results: After adjusting for risk factors of preterm birth (age at conception, education, smoking during pregnancy, and marital status), there was no difference between unintended and unwanted pregnancies compared to intended ones (OR: 1.12, 95% CI: .77 – 1.64; OR: 0.93; 95% CI: .55 – 1.57, respectively). Women having an intermediate level of happiness were significantly more likely to have a preterm birth (OR: 1.95, 95% CI: 1.03 – 3.69).

Conclusions: There was no difference in terms of risk of preterm birth in unintended and unwanted pregnancies compared to intended pregnancies. In terms of happiness, women who reported lower levels of happiness had a higher risk of preterm delivery and vice versa. Happiness of a pregnancy may be a better indicator of adverse birth outcomes since happiness may affect a woman's health behaviors, which ultimately affect birth outcomes.

Sponsor: *N/A*

1509 *(Poster)*

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LESSONS LEARNED: PRIMARY PREVENTION AND RESEARCH WITH HISPANIC FAMILIES.

Purpose: The Primary Prevention Program for Hispanic Families at Risk for Obesity, Type 2 Diabetes (T2D), Metabolic Syndrome, and Cardiovascular Disease (CVD) aimed to encourage children and their families to embrace a healthier lifestyle to achieve a healthy weight and thereby decrease their risk for chronic illness.

Methods: The authors will share lessons learned during the planning and implementation stages of the study. Useful recommendations for designing, planning, and implementing research and interventions among unique populations will be discussed.

Results: Lessons learned highlight 10 issues: recruitment, retention, competing priorities, cultural/linguistic responsiveness, location of intervention/assessment activities, follow-up/locating participants, adults vs. children needs/expectations, and flexibility of research team. Issues such as quality of life, competing priorities and unmet health care needs will take precedence over abstract concepts like prevention. The lessons are significant as they demonstrate extensive time, material and human resources and effort required for successful implementation of interventions among minority populations. Despite vast research experience as well as their experience serving Hispanic populations, the team found recruitment, data collection, and retention of participants more challenging than expected.

Conclusions: Conducting primary prevention interventions and research among minority communities poses multi-dimensional challenges while presenting unique opportunities for multi-disciplinary teams. Research interventions must be amenable to different ethnic groups, gender and lifestyle preferences. To facilitate recruitment, enhance retention and optimize outcomes interventions and research teams must allow for flexibility given the role of cultural, social, and economic factors as determinants of availability, accessibility, and readiness in a special population.

Sponsor: *CDC H75CCH224064*

1510 *(Poster)*

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JOB PRESSURE IN THE CAUSAL PIE CONSTELLATION BETWEEN STRESS AND HEALTH

Purpose: To explore the association between job pressure and musculoskeletal disorders (MSDs) among police officers.

Methods: A descriptive cross-sectional survey was used to collect information on contributing health and lifestyle characteristics to job pressure and the prevalence of MSDs. A standardized Job Stress Survey developed by Spielberger and Vagg (1999) and a customized Health and Lifestyle Questionnaire were used for data collection. The self-administered anonymous surveys were administered across a convenient sample of Dallas Police Officers (n=150). Job pressure was assessed through the Job Pressure Index (JP-X) score. This is a weighted mean of Job Pressure Severity (JP-S), and Job Pressure Frequency (JP-F) subscales.

Results: Seventy valid surveys were analyzed (46.7%). Among these 77.6% were male respondents. In this sample 72.9% were married, 11.9% divorced/separated and 15.3% never married/single. Current alcohol consumers comprised 69.1% and they consume about two drinks a day for about two days each week. The mean (standard deviation) of the JPX score was 27.4(14.5). The overall prevalence of MDS in this sample was 78.6%. Neck problem was reported by 58.5% and headache by 70.8%. The mean JPX score for those who reported neck problem (p=0.01) and headache (p=0.01) were significantly different from the mean scores of those without these complaints. JPX score was also associated with marital status (p=0.04). Job pressure severity was related to self-perceived stress and (p=0.03)

Conclusions: In this sample of police officers, job pressure and MSDs are prevalent. Job pressure seems to be causally related to neck problem and headache. A significant association seems to exist between job pressure severity and self-perceived stress. A good proportion of these officers believe their stress is not under control. An exploratory study to assess the use of manipulative therapy as stress intervention protocol for police officers may be useful

Sponsor: *Parker College Research Institute, Dallas*

1511 *(Poster)*

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Department: Select a Department

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PARENTAL AWARENESS AND UNDERSTANDING OF OVER THE COUNTER PRODUCTS CONTAINING ACETAMINOPHEN (TYLENOL) IN CHILDREN UNDER THE AGE OF 5

Purpose: Acetaminophen (APAP) is widely used among the pediatric population, and although it is a valuable antipyretic and analgesic, studies have shown it has potential liver toxicity. The purpose of this study was to investigate if parents were aware of which common pediatric OTC products contain APAP, co-administration of these products, and potential toxic effects of APAP when given above the recommended dose.

Methods: This survey study was conducted at the UNTHSC Pediatric clinic. A total of 71 primary caregivers who had children under the age of 5 were recruited. Six common OTC product boxes were presented to aid in answering the questions. Parental awareness and understanding were measured by 2 sets of questions: 1) knowledge of APAP, including: which products contain APAP, co-administration of APAP products, and toxic effects of overdosing on APAP and 2) whether or not the participants checked the back of the box for ingredient information. SPSS (12.0) was used to perform ANOVA, x2, and the t-test.

Results: A total of 70 valid surveys were included in the data analysis. The majority of participants were female (88.6%), Hispanic (48.6%), at the age 18-29 (80%), and had a high school diploma or GED as the highest level of education (62.9%). The participants correctly identified 73.2% of the products that contain APAP while observing only 45.5% of the labels to answer this question. Ninety percent of the participants stated that they would not co-administer two products that contain APAP, however, only 25% of the participants looked at the labels while answering these questions. Only 15.9% of the participants knew they would not co-administer the two products because "they both contained APAP." An additional 39% stated "they contained the same medicine," but did not specifically state that APAP was the ingredient. Thus, the remaining 45% of the participants did not understand the reason to not co-administer these products.

Conclusions: The results showed that the caregivers had a good knowledge of the products that contain APAP, but only a superficial understanding of co-administration of these products. There still appears to be a lack of knowledge of potential toxicity of products that contain APAP. Healthcare providers should not assume that their patients are aware of APAP's proper dosing and potential toxicity. Therefore, the healthcare providers should spend some time to explain proper dosing and potential toxicity to all parents of young children.

Sponsor: *N/A*

1512 *(Oral)*

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HEALTH OUTCOME ASSOCIATED WITH THE EXPOSURES OF LEARNING THERAPEUTIC MANIPULATIONS

Purpose: To estimate the burden of the adverse health effects experienced by chiropractic students while learning manipulative therapy.

Methods: The study employed a cross-sectional epidemiological design using self-administered survey. Questionnaires were handed to 890 duly registered students at all levels of the Doctor of Chiropractic program at Parker College of Chiropractic in Dallas. Survey questions were adopted from the Standardized Nordic and Outcome Assessment Health Status Questionnaires. Data were collected on the prevalence and perceived effects of musculoskeletal injuries associated with receiving or administering therapeutic manipulations.

Results: The response rate was 64.3% including 62.6% males. The prevalence of injuries sustained as a result of receiving manipulations from peers was 27.3%. This was most common to the neck/shoulder with a prevalence of 70.7% and a percent attributable risk of 80%. The prevalence of injuries due to administering manipulations was 26.6%. This was most common to the hand/wrist with a prevalence of 67.9% and a percent attributable risk of 98%. The female students were more likely to report injuries than the males [OR =1.45, 95%CI = (1.01, 2.05)]. As a result of injuries they sustained in college, 50.1% of the students made some changes in their manipulative procedures.

Conclusions: A significant amount of musculoskeletal injuries were identified in this study sample. Most of the neck/shoulder injuries were attributable to receiving manipulations from inexperienced hands while the hand/wrist injuries were mostly attributable to administering amateurish manipulations. The actual mechanisms and risk factors to these injuries are unknown. However, some ergonomic factors and proper technique standards may be considered to help prevent them. This study identifies an important need to design a more elaborate study to investigate the incidence of musculoskeletal injuries among students performing therapeutic manipulations across various programs.

Sponsor: *Parker College Research Institute, Dallas*

1513 *(Poster)*

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COMMUNITY HEALTH CENTER USE AS A PREDICTIVE FACTOR OF SATISFACTION WITH CARE AMONG HISPANIC IMMIGRANTS

Purpose: This research will describe community health center (CHC) utilization among Hispanic immigrants and will examine CHC use among Hispanic immigrants as a predictive factor of satisfaction with health care and with cultural competency of care.

Methods: The study analyzes data from the 2001 Commonwealth Fund Health Care Quality Survey conducted via telephone. The study population was a nationally-representative sample of 6,306 adults age 18 and older. Univariate and bivariate statistical analyses provide descriptive statistics about CHC utilization among foreign-born and U.S. born Hispanics. Using a data subset of foreign-born Hispanics who use CHCs as their usual source of care, separate multivariate logistic regression analyses will predict satisfaction with cultural competency of services and with medical care received.

Results: The anticipated results of this study will show what percentage of the Hispanics who use CHCs as their usual source of care are foreign-born and if those who utilize a CHC are less likely to delay seeking care than those who use another source of care. Results will also indicate whether foreign born Hispanics are more likely to be satisfied with medical care at a CHC than that from another source of care, as well as show whether foreign-born Hispanics are more satisfied with the cultural competency of care provided at a CHC, compared to other sources of care.

Conclusions: Results will inform current policy recommendations for CHC expansion as a means to improve health care for the growing population of Hispanic immigrants.

Sponsor: *N/A*

1514 *(Poster)*

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THE ROLE OF PHYSICAL ABUSE AND ALCOHOL ON MALE AND FEMALE DEPRESSION AMONG COUPLES IN THE U.S.

Purpose: This study investigated the association of depression with physical abuse and alcohol use and related problems in a representative sample of U.S. couples who were part of a longitudinal study.

Methods: Analyzed were 1136 couples of the same ethnic background who were still together at follow-up (406 Caucasian, 387 Hispanic, 232 African-American, and 111 mixed/other). Regression analyses were used to identify physical abuse and alcohol variables as predictors of depression while controlling for sociodemographics and other covariates.

Results: Overall, women showed a higher prevalence of depression (11.5%) compared to men (7.8%). Female alcohol-related problems (OR= 3.15; 95% CI= 1.07-9.23) and female-perpetrated physical abuse (OR= 3.01; 95% CI= 1.24-7.29) predicted female depression. Women reporting drinking, without binging, were less likely to experience depression. Women who had been victimized only or couples who reported mutual violence did not experience increased odds of depression. Presence of abuse and men's drinking did not predict depression among men. Although not significant, increased odds (OR=2.92; 95% CI=0.70-12.18) were seen in those men with a couple-reported male-to-female violence only.

Conclusions: The findings highlight the need for continued focus on depression in women among couples involved in physical violence. Further study should be given to the role of depression in both the victim and the perpetrator of physical violence.

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1515 *(Poster)*

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UTILIZATION OF THE BALANCED SCORECARD FRAMEWORK: PATIENT SATISFACTION, EMPLOYEE SATISFACTION, AND FINANCIAL METRICS IN A SOUTHWEST HOSPITAL

Purpose: In this retrospective study, the authors identified lessons learned from the Balanced Scorecard (BSC) framework's use in an intervention entitled Route 99 (R99). R99 occurred from January to December of 2003 at a Southwest hospital. Patient Satisfaction (PS), Employee Satisfaction (ES), and financial metrics were utilized to identify these lessons.

Methods: Individual PS surveys were administered and compiled by an outside vendor. As the driving metric for R99, quarterly mean PS scores for inpatient and outpatient populations were examined through one-way Analysis of Variance (ANOVA) utilizing SPSS. The ANOVA compared PS by four time intervals, pre full-time employee reduction (Pre-FTER, n=10), full-time employee reduction to R99 (FTER-R99, n=10), R99 (n=4), and post R99 (n=2). ES was evaluated through a five point likert scale, twelve question employee survey administered at the beginning of R99 (n= 227) and seven months into R99 (n= 191). The surveys were compared with the Mann-Whitney U Test. Debt ratio, days cash on hand, total profit margin, and outpatient growth rate were estimated from the hospital's annual reports and examined for any trends.

Results: Significant increases ($p < 0.05$) for outpatient mean PS scores were observed for R99 as compared to Pre-FTER and FTER-R99. A significant decrease ($p < 0.05$) in inpatient mean PS scores from Pre-FTER to FTER-R99 was identified as well. Significant decreases ($p<0.05$) in three ES questions were detected in the Mann-Whitney analyses. The debt ratios of 0.63 to 0.84 from 2001 to 2003 reflected the decreasing assets and the increasing debt of the hospital. Days cash on hand trended downward from 1999 to 2002 with 129.2 and 57.3 days, respectively. The outpatient department growth rates of 9.5 in 1998 to 13.5 in 2001 were followed by sharp declines ending at -8.1 in 2003. Total profit margin fluctuated but did not demonstrate a trend.

Conclusions: Increased outpatient PS scores were attributed to the BSC framework intervention, while decreased ES was attributed to financial turmoil. The inverse relationship was in contrast to which has been reported in the literature. The debt ratio indicated financial problems prior to R99. Given the BSC framework's success despite counter indications led the authors to two lessons. First, PS may not have been the best indicator to drive R99. Second, the BSC framework intervention may have been more productive directed at the hospital's financial issues.

Sponsor: *N/A*

1516 *(Poster)*

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Presenter: Alberto Coustasse

Department: School of Public Health

Classification: Faculty

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PILOT STUDY IN UNCOMPENSATED CARE IN A SAMPLE OF TEXAS HOSPITALS

Purpose: This descriptive study examined uncompensated care in United States, Texas, and Tarrant County hospitals, and the methods which hospitals are reimbursed for care provided, as well as policy implications of uncompensated care.

Methods: To identify uncompensated care charges, costs, and reimbursement, data was utilized from four sources: 1) the US/Mexico Border Counties Coalition 2002 report: Medical Emergency Cost of Uncompensated Care in Southwest Border Counties; 2) the 2004 Texas Hospital Association Survey compiled by the Center for Health Statistics within the Texas Department of State Health Services; 3) the CMS report: Federal Reimbursement of Emergency Health Services Provided to Undocumented Aliens FY 2006; and 4) the 2004 Texas Health and Human Services Commission's report for Texans' health insurance coverage.

Results: Costs due to undocumented immigrants in border county hospitals were highest in San Diego County, CA, El Paso County, TX, and Hidalgo County, NM with $76,185,000, $30,102,000, and $19,666,000, respectively. Of uncompensated care dollars, Arizona border counties displayed the highest percentage due to undocumented aliens at 31.7%, while Texas presented the lowest with 17.9%. Reimbursements from CMS were led by California and Arizona with $66,641,038 and $47,650,474, respectively. Arizona's CMS reimbursement was largely attributable to higher apprehension of illegal immigrants. Tarrant county hospitals generated $933,794,289 in uncompensated care charges, of which about $500 million were originated in charity expenditures.

Conclusions: The definition of uncompensated care is inconsistent within the literature and between agencies. The inability to clearly define uncompensated care has complicated legislature's ability to provide funding and create reimbursement channels. Additionally, uncompensated emergency services have implications beyond loss of hospital revenues such as rising health care costs and insurance premiums which threaten business' ability to offer employees affordable health care benefits. High costs and low levels of reimbursement are threatening the viability of ERs and public hospitals around the nation. This study has demonstrated the continued escalation of the uncompensated care crisis with its multitude of financial, social, and health ramifications. While the Medicare Modernization Act has attempted to address the increasing imbalance, the shortfall of funds highlights the growing crisis and need for policy intervention.

Sponsor: *N/A*

1517 *(Poster)*

Author: Katandria Johnson

Presenter: Katandria Johnson

Department: School of Public Health

Classification: SPH Student

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HEALTH DISPARITIES AMONG MEXICAN IMMIGRANTS IN THE UNITED STATES

Purpose: It is hypothesized that challenges faced by the United States healthcare system regarding the provision of cost-efficient, immigrant healthcare services is due to a disparity of healthcare services, a dearth of bilingual and bicultural healthcare professionals and increased healthcare costs.

Methods: Literature reviews, case studies and internet resources were utilized for this research, as provided by the UNTHSC Gibson Lewis Library.

Results: The type of research being conducted on health disparities includes: 1) projects aiming to identify disparities; 2) identification of causes; 3) identification of which causes have the greatest impact on quality of care and health outcomes; 4) ways to address the disparities; and 5) the impact of and effectiveness of programs (Exworthy and Washington, 2006). Types of research being conducted may be influenced by Healthy People 2010 because of its aim to reduce health disparities among six target diseases including diabetes, cardiovascular disease, infant mortality, HIV/AIDS, cancer screening and prevention, and immunization (Healthy People; Exworthy and Washington, 2006). Such research resulted in more funding in these areas and a larger number of studies are being conducted in these six areas.

Conclusions: Program curriculums should include research that addresses the availability and efficacy of existing healthcare services to immigrant populations. Cross-cultural, comparative research that examines the ethnic and cultural beliefs regarding healthcare decisions among this patient population is of equal importance. Research data could be obtained through national statistical offices and the United States census bureau towards the development of a community program or household survey designed to collect information about disability issues and access to healthcare services. In addition, the training of personnel in general fields such as social assistance, public health, medicine, education, and vocational rehabilitation can be executed through local or international mentor programs and continuing education teleconferences. Increased sociocultural and sociolinguistic content in undergraduate and graduate coursework, practicum, or residencies is also recommended; as well as study abroad options for service professionals who desire to increase their linguistic and cultural competence.

Sponsor: *N/A*

1518 *(Oral)*

Author: Ana Luz Chiapa
Presenter: Ana Luz Chiapa
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DIFFERENCES IN PREGNANCY INTENTIONS AND BIRTH OUTCOMES: A COMPARISON OF BLACK, HISPANIC, AND WHITE WOMEN

Purpose: The purpose of this study was to understand how pregnancy intendedness and level of pregnancy happiness impact birth outcomes among Black, Hispanic, and White women in the United States.

Methods: This study utilized the National Survey of Family Growth Cycle 6. This survey is designed and administered by the National Center for Health Statistics (NCHS). The sample for this study included White, Hispanic and African American women who were 20 years of age and older. The dependent variable for this study was preterm birth. The two independent variables of interest were pregnancy intendedness and pregnancy happiness. An intended pregnancy is one that occurs at the right time or later than wanted. Unintended pregnancies are those that are mistimed or unwanted pregnancies. Happiness of being pregnant is measured as a scale from 1 to 10, with 1 being very unhappy to 10 being very happy.

Results: When the association between preterm birth, and intendedness was adjusted for risk factors of preterm birth (age at conception, education, smoking during pregnancy, and marital status), there was no difference between unintended and unwanted pregnancies compared to intended ones. Women having an intermediate level of happiness were significantly more likely to have a preterm birth (OR: 1.95, 95% CI: 1.03 - 3.69). When the model was stratified by race, White women who reported being unhappy about their pregnancy had 3.97 times the odds of a preterm delivery (95% CI: .56 - 28.12), and Black women had 4.01 times the odds (95% CI: .68 - 23.61), compared to those who reported being very happy. Moreover, Black women who had an intermediate level of happiness had 2.45 times the odds of preterm delivery (95% CI: .95 - 6.30).

Conclusions: After controlling for risk factors, there were no differences in risk of preterm birth among White, Black and Hispanic women comparing unintended or unwanted pregnancies with intended ones. However, Black women who reported being unhappy about their pregnancy had a higher risk of preterm delivery than White or Hispanic women who reported being unhappy, compared to those who reported being very happy. Happiness may be a better predictor of adverse birth outcomes and may help to better explain health disparities in this area. It may also prove useful for health care providers, as it can help identify areas in which pregnant women may need more help in order to have a healthy baby, such as smoking cessation.

Sponsor: *N/A*

1519 *(Poster)*

Author: Jill Estep
Presenter: Jill Estep
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WHAT FACTORS INFLUENCE THE INTENT TO OBTAIN TRIPLE OR QUAD SCREEN TESTING FOR DOWN SYNDROME IN NON-PREGNANT WOMEN AGES 18-45?

Purpose: The triple screen and the newer quad screen are tests that can be used to determine if a pregnant woman is at high risk for having a baby with Down syndrome. This study investigated what factors, including the Influence of Others, Down Syndrome Experiences, Cost, and Personal Factors, would most influence a woman's decision to obtain triple or quad screen testing for Down syndrome if she were to become pregnant.

Methods: A 13-question Likert scale survey was developed and pilot tested. The survey included brief information on Down syndrome and the triple/quad screen test, 6 demographical questions, and one question on whether the woman would likely choose to have triple/quad screen testing if she were pregnant. After gaining UNTHSC IRB approval, the survey was handed out to 78 non-pregnant, English-speaking literate patients between the ages of 18 and 45 at Brazos Medical Associates clinic in College Station, TX. Descriptive statistical analysis, logistic regression analysis, and one-way ANOVA analysis were performed using SPSS (14.0) software.

Results: The data showed that the Influence of Others category was rated as the most influential factor (3.12/4) with "partner's opinion" and "doctor's recommendation" being the top two highest scoring factors overall (3.51/4 and 3.50/4, respectively). Cost received the lowest score of all (2.28/4). However, the Personal Factors category was the only category that statistically significantly predicted whether a woman would choose testing ($p = .001$). Within this category, it was "personal anxiety" that statistically significantly predicted test desire ($p = .001$).

Conclusions: There are many factors that a woman would highly consider in her decision to obtain triple/quad screen testing, such as her partner's and doctor's opinions. However, the only predictor of whether a woman would actually seek testing was her own perceived anxiety. It can thus be concluded that healthcare providers should not only cover the medical aspects of prenatal genetics testing in their conversations with patients and their partners, but should also take into consideration the woman's emotional and psychological status in order to help alleviate any unnecessary anxiety. This study was limited by selection and site bias as well as by the design of the study. As a result, the conclusions cannot be accurately applied to the general female population.

Sponsor: *N/A*

1520 *(Poster)*

Author: Julius Larry III

Presenter: Julius Larry III

Department: Health Management and Policy

Classification: SPH Student

Julius J. Larry III-SPH student; Patricia Schlorke-Dr.PH student; Alberto Coustasse- Principal Investigator-SPH; Kristine Lykens, HMAP-SPH; Sue Lurie-SPH

PILOT STUDY OF KAWASAKI DISEASE IN TEXAS REGIONS: PUBLIC HEALTH CONSIDERATIONS

Purpose: The purpose of this retrospective cross-sectional study was to evaluate Kawasaki Disease in Texas children by age at onset; race/ethnicity; gender; co-morbidities and Public Health region.

Methods: The study sample included 307 Kawasaki Disease cases in Texas in 2004 from a data population of 2,818,460 hospital discharges obtained from the Texas Hospital Inpatient Discharge database. The 2004 database was sorted by Principal Diagnosis and ICD-9 code to obtain all reported Kawasaki Disease cases. Public Health Regions were used as the dependent variable. An evaluation of incidence per region; race; ethnicity; gender; patient age and length of hospital stay was performed.

Results: Results revealed that Public Health (PH) Region 3, which included Dallas and Tarrant Counties, presented the highest incidence of reported Kawasaki Disease cases in Texas in 2004; followed by PH Region 6, which included Galveston and Harris Counties. Public Health Region 11, which included Bee and Hidalgo Counties, experienced the third highest incidence of Kawasaki Disease in Texas in 2004. Ages ranged from 29 days to 39 years. Average length of hospital stay was 4.4 days. All races were affected. The male-female ratio was 1.58:1.

Conclusions: The Public Health Regions in Texas were unaware of the number of Kawasaki Disease cases in their regions in 2004 due to the lack of active surveillance of the disease. No statewide database is currently maintained nor are accurate numbers of Kawasaki Disease cases every year. The results further suggest the fact that the cardiac sequelae of Kawasaki Disease, including coronary artery aneurysms, affect adolescents and adults due to untreated or unrecognized disease in childhood. Active surveillance is needed to learn more about etiology that could lead to the discovery of the pathologic agent. The national Kawasaki Disease surveillance system is an important tool to monitor the possible occurrence of nationwide outbreaks and to monitor the trend of cardiac complications among all Kawasaki Disease patients and specific racial and ethnic groups.

Sponsor: *N/A*

1600 *(Poster)*

Author: Angela Goetz

Presenter: Angela Goetz

Department: Pharmacology & Neuroscience

Classification: GSBS Student

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RECIPROCAL MUTATIONS IN THE SECOND TRANSMEMBRANE DOMAIN OF THE D2 AND D4 DOPAMINE RECEPTOR DIFFERENTIALLY AFFECT THE BINDING OF THE ANTIPSYCHOTIC DRUG ARIPIPRAZOLE AND ITS DERIVATIVES

Purpose: Dopamine receptors consisting of five genotypically distinct subtypes (D1, D2, D3, D4 and D5) are implicated in the pathology or treatment of numerous neurological and psychiatric disorders. Since the receptor subtypes show distinct pharmacological properties, it is of general interest to discover subtype selective compounds and to understand how they recognize their receptor. Our goal is to understand the receptor-ligand interaction of D2- and D4-selective compounds. One compound used in our studies is aripiprazole, a new generation antipsychotic approved for the treatment of schizophrenia. In this study we determined the binding affinities of aripiprazole and three structurally related compounds for the D2 and D4 wild type (WT) receptors and for two receptor mutants. The mutants are characterized by a reciprocal mutation in the second transmembrane domain of the D2 and the D4 receptor. Previous work from our lab demonstrated that this mutation in the D4 receptor increases the binding affinity of some D2-selective compounds.

Methods: WT and mutant receptors were expressed in HEK293 stable cell lines. Ligand affinities were determined by competitive binding experiments using [3H]N-Methylspiperone as radioligand.

Results: Ligand binding revealed that the moderately D4-selective compound LB109 shows highly increased affinity for both mutations compared to the WT receptors. However, MS195, a derivative of LB109, shows no subtype selectivity, but is also sensitive to the mutations. Aripiprazole, a D2-selective antipsychotic drug, shows a D2-like affinity increase for the mutant D4 receptor. MS169, a derivative of aripiprazole, shows less D2-selectivity, but also displays a D2-like affinity increase for the D4-mutant. However, both compounds are not sensitive to the D2-receptor mutation.

Conclusions: Ligand binding experiments showed that the moderately D4-selective compound LB109 and the non-selective compound MS195 are sensitive to the corresponding mutation in both D2 and D4 receptor backgrounds, whereas the D2-selective compounds aripiprazole and MS169 are only sensitive to the mutation in the D4 receptor background. Computer-based molecular models based on the crystalline structure of rhodopsin predict that the mutated amino acid is not located in the binding site crevice. This raises the possibility that the observed effects may be allosteric in nature.

Sponsor: *NIH R01 MH063162 (JAS)*

1601 *(Poster)*

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Presenter: Andrew Payne

Department: Pharmacology & Neuroscience

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CREATION OF A DOPAMINE RECEPTOR – YELLOW FLUORESCENT PROTEIN FUSION CONSTRUCT

Purpose: Dopamine receptors have been implicated in a variety of normal physiological processes and the treatment of neurological and psychiatric disorders. A powerful tool to aid the study of dopamine receptors would be the ability to visualize the protein in mammalian cells. The goal was to fluorescently label the human dopamine receptor using molecular biology techniques. Previous studies identified intracellular loop 3 (IC3) of the D2 dopamine receptor as a hyper variable domain, suggesting that IC3 can be modified without significantly changing the receptors properties. Yellow Fluorescent Protein (YFP) is a 130 residue protein that absorbs UV light and emits a yellow visible light. The inserted fluorescent label will be a useful tool in further studies of receptor function and trafficking in live cells.

Methods: DNA was cut by restriction enzyme digestion and fragments were isolated by agarose gel electrophoresis. Comparisons by size and banding patterns generated by restriction digest were used to determine sequences of interest. Recovered fragments were ligated with T4 DNA Ligase, transformed to E. coli bacteria, and selected with a drug resistance marker. Recombinant plasmids were purified from E. coli.

Results: Insertion of the YFP into D2 has been verified by DNA sequencing. Restriction digest and agarose gel electrophoresis of the hybrid gene displays a molecular weight equal to the predicted D2 + YFP.

Conclusions: The D2 receptor has been genetically modified with a YFP marker. Future work includes expression of the fusion protein in mammalian cells.

Sponsor: *NIH R01 MH063162 (JAS)*

1602 *(Oral)*

Author: David Cummings

Presenter: David Cummings

Department: Graduate School of Biomedical Sciences

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MODIFICATION OF A SUBTYPE SELECTIVITY MICRODOMAIN IN THE D2 DOPAMINE RECEPTOR CHANGES THE AFFINITY AND FUNCTION OF L750,667

Purpose: The dopamine receptor, particularly the D2 subtype, has been a major focus of pharmacological research due to its prominent roles in schizophrenia and Parkinson's disease. The D4 receptor is a homologue that shows promise as a target for the treatment of erectile dysfunction and possibly attention deficit disorder. Understanding the molecular mechanisms behind selective drug affinity and potency is crucial for designing drugs with highly specific pharmacology. We tested the subtype selectivity of the D2 receptor by mutating three amino acid residues to the corresponding residues in the D4 receptor and then probed the mutant receptor with the D4-selective antagonist L750,667.

Methods: To obtain cells expressing wild type or mutant D2 receptors, HEK293 cells were transfected with the corresponding plasmid DNA, grown under selective pressure, isolated, and tested for expression. Receptor affinity for L750,667 was determined by competition with [3H]methylspiperone. Functional assessment of L750,667 was determined by a cAMP competition assay and reported as a percentage of maximal cAMP.

Results: Radiolabeled competition showed that the mutant receptor has a greatly increased affinity for L750,667 in comparison to the wild type. The potency of (-)-quinpirole was similar in both the wild type and mutant receptors but a single concentration of L750,667 was only able to elicit a decrease in potency for the mutant receptor. L750,667 was found to have a partial agonist response at the wild type receptor but, unexpectedly, an antagonistic response at the mutant receptor.

Conclusions: We find that the mutant receptor has a greatly increased affinity for L750,667 in comparison to the wild type receptor. Functionally, we find that L750,667 is an agonist at the wild type receptor but changes to an antagonist at the mutant receptor. The findings indicate that these three amino acids are important for the affinity and function of L750,667 and probably other compounds with similar structure.

Sponsor: *NIH R01 MH063162 (JAS)*

1603 *(Oral)*

Author: Diana Canseco

Presenter: Diana Canseco

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Classification: GSBS Student

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SYNTHESIS OF NOVEL SUBSTRATES AS POSSIBLE SEROTONIN RECEPTOR LIGANDS

Purpose: Serotonin (5-hydroxytryptamine) is a neurotransmitter that plays a major role in the regulation of mood, sleep, emesis, sexuality, and appetite. Fourteen distinct forms of serotonin receptors have been identified and are grouped into seven subfamilies (5-HT1 through 5-HT7) based on gene structure, amino acid sequence homology and intracellular signaling cascades. Efforts to develop agonists that can distinguish specifically between the clinically relevant 5-HT2A and 5-HT2C serotonin receptor subtypes have been challenging as the 5HT2A and 5-HT2C receptors sequences are highly conserved, especially in receptor microdomains that are considered important for agonist binding. Previous work on natural products isolated from marine sources identified a pharmacophore that was selective for the 5-HT2C subtype, but that had low affinity. Our goal is to exploit this natural product pharmacophore in an effort to create derivative compounds that have improved affinity for the 5-HT2C receptor. Our hypothesis is that the affinity and selectivity for the human 5-HT2C receptor subtype will be enhanced in aplysinopsin derivatives with halogen atoms in the 5 position of the indole ring.

Methods: Novel indoleamines were designed based upon structurally related compounds that have been isolated from a variety of marine species, including the sea slug Aplysia. The so called aplysinopsin derivatives were synthesized by carrying out an aldol condensation with 5-chloro- or 5-bromoindole-3-carboxaldehyde and creatinine. The structures of all compounds synthesized were verified using spectroscopic methods.

Results: Two aplysinopsin-like derivatives have been synthesized. Their structures have been verified by proton and carbon nuclear magnetic resonance spectroscopy. A single crystal X-ray structure on one of these compounds has been carried out. Work is in progress on the synthesis of the indole aldehydes needed for the synthesis of other aplysinopsin derivatives.

Conclusions: Two new 5-halogenated aplysinopsin derivatives have been synthesized and their structures characterized. The X-ray crystal structure on one of them shows that the indole ring is oriented on the same side as the carbonyl group in the creatinine ring.

Sponsor: *G67673(JAS), R25GM55380(JEJ), Welch Foundation M0200(JEJ), and TWU Research Enhancement Program(JEJ)*

1604 *(Oral)*

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Presenter: Lorie Gonzalez

Department: Pharmacology & Neuroscience

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A MECHANISTIC STUDY OF CARISOPRODOL ACTIONS AT $GABA_A$ RECEPTORS

Purpose: Given the present and potential danger posed by carisoprodol abuse, it is of crucial importance to determine the mechanism of action of this drug. The sedative effects of carisoprodol are believed to be due to its metabolite, meprobamate—a controlled substance at the federal level. In contrast, we have provided evidence that carisoprodol, itself, is capable of directly activating and allosterically modulating $GABA_A$Rs in a barbiturate-like manner. To gain further insight into the mechanism and site of action of carisoprodol, we have assessed its potential subunit- and receptor-dependent interactions.

Methods: Whole-cell patch clamp electrophysiology was utilized to investigate carisoprodol-mediated effects on $GABA_A$R function. In order to investigate whether the direct effects of carisoprodol are dependent upon the α subunit isoform, currents were recorded from human embryonic kidney 293 (HEK 293) cells stably expressing either rat α1β2γ2 or α6β2γ2 $GABA_A$Rs. Barbiturate-insensitive ρ1 GABA subunits were converted to barbiturate-sensitive ρ1(W328M) subunits via site-directed mutagenesis. To confirm the gain of function, HEK 293 cells were transfected with homomeric ρ1(W328M) receptors and tested for sensitivity to pentobarbital. Using the same experimental model, currents were recorded from homomeric ρ1(W328M) receptors to investigate whether the mutation conferred allosteric or direct sensitivity to carisoprodol.

Results: In whole-cell patch clamp recordings, carisoprodol was significantly more efficacious at directly activating α6β2γ2 compared to α1β2γ2 receptors, indicating the direct effects of carisoprodol are influenced by the α subunit isoform. In contrast to αβγ receptors, homomeric ρ1 GABARs are insensitive to barbiturates. We found they are similarly insensitive to carisoprodol. We confirmed a previous report showing the mutation W328M can confer barbiturate sensitivity to ρ1 GABAR. Interestingly, the same mutation could not confer sensitivity to either the allosteric or direct effects of carisoprodol.

Conclusions: Whereas the functional effects of carisoprodol are comparable to barbiturates, our results indicate the binding domains for the two ligands are not equivalent. Further, the ability of carisoprodol to directly activate $GABA_A$Rs lends support to the contention it should be a scheduled drug.

Sponsor: *N/A*

1700 *(Oral)*

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Presenter: Archit Gulati

Department: Pharmacology & Neuroscience

Classification: GSBS Student

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DEVELOPMENT OF A BIOCHEMICAL ASSAY TO SCREEN FOR COMPOUNDS THAT POTENTIALLY INHIBIT MUSSEL GLUE-PROTEIN ACTIVATION

Purpose: Zebra mussels are known to produce a glue protein that becomes adhesive through an enzymatic reaction involving tyrosine and an enzyme with tyrosinase activity. The purpose of this study is to develop biochemical assays, in order to test whether compounds previously identified as having antifouling activity also inhibit tyrosinase activity.

Methods: A microtiter colorimetric assay was developed for detecting 3,4-dihydroxyphenylalanine (DOPA), which is only present in the activated structure of the glue protein. A standard curve was created using the reaction of DOPA with nitrite. Next, the ability of a tyrosinase enzyme to convert tyrosine to DOPA was quantified. In order to validate the assay, this reaction was then evaluated in the presence of known inhibitors of tyrosinase.

Results: An accurate colorimetric assay was developed for detecting DOPA in solution. Using a DOPA standard curve, the presence of DOPA in activated glue protein in solution was accurately predicted. Known inhibitors of tyrosinase prevented the conversion of tyrosine to DOPA.

Conclusions: The assays developed in this study will be useful for determining whether antifouling compounds inhibit tyrosinase. This will allow us to investigate possible biochemical mechanisms of zebra mussel antifoulants.

Sponsor: *G67673 (JAS), G67699 (JAS, RFM) and G68877 (JAS, RFM)*

1701 *(Poster)*

Author: Tarig Alkhazin

Presenter: Tarig Alkhazin

Department: Pathology and Human Identification

Classification: GSBS Student

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DNA EXTRACTION OF FORENSIC CASEWORK SAMPLES USING THE MAXWELL® 16 ROBOTIC PLATFORM

Purpose: A new and automated instrument by Promega™, known as Maxwell® 16 DNA extraction system, is being validated for the processing of forensic casework samples. The Maxwell® 16 extracts DNA by utilizing reagents and paramagnetic particles in the pre-filled cartridge format of DNA IQ™ Casework Sample Kits. An entire Maxwell® 16 run takes approximately 20 minutes, during which DNA from up to 16 samples can be extracted, purified, and finally concentrated in a low elution volume. The goal of this validation was to measure the reliability of the Maxwell® 16 in extracting DNA from common forensic case work samples in order to amplify the 13 core CODIS (Ccombined DNA Index System) STR (Short Tandem Repeat) loci for forensic DNA analysis.

Methods: To measure performance, the Maxwell® 16 was rigorously tested on its reliability in extracting DNA from various samples that include different blood dilutions as well as various tissue samples such as muscle, skin, and hair. In addition, trace samples were also prepared by swabbing empty soda cans. Replicates of samples were processed by both the Maxwell® 16 and by current lab procedures for organic extraction in order to compare results. DNA was quantitated using Real-time PCR (Polymerase Chain Reaction) and electropherograms of STR profiles were produced.

Results: Amplifiable DNA was obtained from all samples and by both methods. However, DNA quantitation results revealed that the Maxwell® 16 extracted DNA in a more consistent and reproducible manner than organic extraction. Although it yielded less DNA than organic extraction, the Maxwell® 16 extracted purer, higher quality DNA that proved to be more viable in downstream STR typing. In terms of efficiency, the Maxwell® 16 required less manual labor and processed an equal work load in less time.

Conclusions: The Maxwell® 16 is an automated DNA extraction system that can process forensic casework samples and match current lab procedures in performance. With additional testing, the reliability and the added efficiency of the Maxwell® 16 may be of significant use to forensic labs.

Sponsor: *N/A*

1702 *(Poster)*

Author: Rachel Murff
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Department: Physician Assistant Studies
Classification: TCOM MPAS Student
Rachel Murff, PA-S; Patti Pagels, MPAS, PA-C; Olive Chen, PhD

PHYSICIAN ASSISTANTS' KNOWLEDGE OF MEXICAN AMERICAN BEHAVIORS OF FAMILISM, SIMPATIA, AND MACHISMO

Purpose: While many reports discuss cultural competence among healthcare providers, few evaluate their knowledge of specific cultural behaviors. The objective of this study was to investigate Physician Assistants' knowledge of Mexican American cultural behaviors of familism, simpatia, and machismo.

Methods: The researchers designed a 14-item survey based on previous literature. The survey was pilot tested by 2 Physician Assistant faculty members at the University of North Texas Health Science Center. The survey was distributed electronically to all members of Texas Academy of Physician Assistants(TAPA) and Florida Academy of Physician Assistants(FAPA). Descriptive statistics were used to report the knowledge level of the respondents regarding familism, simpatia, and machismo. SPSS was used to perform ANOVA and t-tests to compare the differences between age, ethnicity, and graduation date on the knowledge level of Mexican American cultural behaviors.

Results: Total number of respondents to the survey was 495. Data was analyzed based on 445 valid data. The results showed that respondents had a high level of knowledge regarding 'familism' (Mean= 2.73/3.00) and 'machismo'(Mean= 2.85/3.00), and a lower level of knowledge regarding 'simpatia'(Mean= 2.40/3.00). The level of knowledge Physician Assistants had regarding Mexican American cultural behaviors had not reached statistical significant differences among different age groups (F=0.244, p=0.913), ethnic groups (F=0.083, p=0.920), graduation dates (F=0.042, p= 0.989), and organization groups (t=-1.451, p=0.148). However, females were found to have a significantly better knowledge than males(t=-2.334, p=0.02)regarding Mexican American cultural behaviors. It was also found that having knowledge of 'familism' corresponded also to having better knowledge of 'simpatia'(r=0.127, p=0.008)but not of 'machismo'(r= 0.042, p= 0.382). Having knowledge of 'simpatia' corresponded also to having better knowledge of 'machismo' (r=0.127, p=0.009).

Conclusions: Physician Assistants displayed a high level of knowledge of 'simpatia', 'familism', and 'machismo. Limitations included lack of email access, nature of True/False survey, and confusion between terms Hispanic and Mexican American. More research needs to be done to determine the degree that Physician Assistants apply their knowledge in practice.

Sponsor: *N/A*

1703 *(Poster)*

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Presenter: Thomas Yorio, PhD
Department: Research
Classification: Faculty
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HEALTH INSTITUTES OF TEXAS

Purpose: The Health Institutes of Texas (HIT) was established in 2007 at the UNT Health Science Center with the intent to provide a bench-to-bedside solution for the people of Texas. The purpose of HIT is to leverage UNTHSC's expertise in public health, interdisciplinary scientific research and clinical care education and delivery to translate public health information and basic research into new models of provider training and care delivery. This will be achieved, in part, through three centers: Texas Center for Health Outcomes, Texas Center for Translational Research and Texas Center for Primary and Rural Care.

Methods: The Texas Center for Health Outcomes will provide our public health experts an all-important information repository to target the exact existence of serious socio-economic and ethnic health disparities, as well as the emergence of new diseases.

Results: The Texas Center for Translational Research will develop new treatment paradigms based on our research strengths in Alzheimer's and aging, health disparities, diabetes, obesity, musculoskeletal medicine, cardiovascular disease, and infant mortality through new interdisciplinary and collaborative investigations and partnerships.

Conclusions: The Texas Center for Primary and Rural Care will take the information from the Centers and develop new models of care that will improve health care in rural and underserved communities in Texas. All three centers working together will provide the continuous feedback necessary to determine if a solution in theory works in reality.

Sponsor: *N/A*

1704 *(Oral)*

Author: Krystle Macurdy
Presenter: Krystle Macurdy
Department: Cell Biology and Genetics
Classification: GSBS Student
Krystle Macurdy, Suzanne Gonzalez, Rhonda Roby, Dr. Arthur Eisenberg and Dr. John Planz

RESEQUENCING OF THE HUMAN MITOCHONDRIAL GENOME TO AID IN HUMAN IDENTIFICATION THROUGH SEPARATION OF THE COMMON CAUCASIAN HAPLOTYPE GROUP

Purpose: In forensics today, the standard approach for human identification is through nuclear DNA analysis. However, there are not always sufficient levels of nuclear DNA to conduct testing; as a result analysts turn to mitochondrial DNA (mtDNA) for identification. To date, the forensic community focuses their analysis on hypervariable region I and II (HVI and HVII) in which most of the variation in humans exists. Common haplotypes observed in the HVI and HVII regions have limited the ability for analysts to differentiate between individuals. The purpose of this study is to conduct whole mitochondrial genome sequence analysis in hopes to further separate the common haplotypes to accomplish better differentiation between individuals of separate maternal lineages. We hypothesize that unique polymorphisms will be identified in areas outside HVI and HVII to enable the separation of individuals sharing the same common haplotype.

Methods: The evaluation of mitochondrial sequence was accomplished using two different systems: the GeneChip® Human Mitochondrial Resequencing Array 2.0 which utilizes DNA hybridization to a microarray chip and the mitoSEQrTM Resequencing System which uses fluorescent-based cycle sequencing reactions with BigDye Terminator v3.1 Cycle Sequencing Kit. This mini-study focuses on 10 individuals whom all share the same haplotype within the hypervariable regions utilized during forensic analysis.

Results: The results of both methodologies identified polymorphisms outside the HVI and HVII regions that are not shared among the 10 Caucasians examined. Also, the results provided the ability to investigate the potential use of microarray technology as a possible alternative to the labor intensive, cycle sequencing technique. More specifically, the results reveal specific limitations of the chip array which include the inability to detect insertions and deletions, false heteroplasmy calls and the presence of numerous ambiguous calls.

Conclusions: The results enable the ability to differentiate between individuals of the same haplotype by expanding analysis to the whole mitochondrial genome. The limitations of the microarray methodology indicate that this approach is not yet ready for forensic analysis, however it does show potential.

Sponsor: *NIJ*

1705 *(Poster)*

Author: Shannon Danner
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THE ATTITUDE OF MEDICAL EXAMINERS TOWARD THE EMPLOYMENT OF PHYSICIAN ASSISTANTS

Purpose: The purpose of this research was to investigate the attitude of Medical Examiners regarding the likelihood of employing a physician assistant (PA) in a Medical Examiner's office (MEO), potential capabilities of PAs, potential economic benefits of using PAs in a MEO, and familiarity with PAs.

Methods: Researchers developed a survey containing 16 statements and 5 demographic questions. The survey was pilot tested on three professionals in the forensic community for clarity and estimated time of completion. After gaining the UNTHSC IRB approval the researchers emailed the survey to approximately 900 members of the National Association of Medical Examiners (NAME). Results were analyzed with descriptive statistics, Chi-square, and stepwise linear regression analysis by SPSS 12.0 software.

Results: The response rate was 7.8% (n=71) with 61 valid responses. The majority of respondents agreed as follows: 1) PAs could work in the capacity in which the survey inquired (x^2=37.441, p=0.001); 2) PAs have the potential capability to work in an MEO (x^2=9.6, p=0.002); 3) PAs could bring economic benefits to an MEO (x^2=12.356, p=0.001); and 4) MEs were familiar with the PA profession and education (x^2=26.667, p=0.001). Furthermore, the majority of the respondents (73%) also agreed that PAs would be an asset in an MEO and believed that PAs could represent the ME at the scene of death (85%); however, only 38.2% of respondents said they would hire a PA in the future. The results also showed that the two major predictors of an ME's plans to employ a PA in the future were potential economic benefit of PAs and the Northeast locale(R=.732, p=0.001 and R=.180, p=0.048, respectively).

Conclusions: This research, one of the first of its kind, provided empirical data to support the outlook that PAs are capable of working in an MEO and would be an asset to an MEO. The economic benefits of PAs appeared to be the most important predictor of whether an ME would hire a PA in the future. Lack of funding may be a reason for less than half of respondents planning to hire a PA in the future. A limitation of the survey is that it was only sent to members of NAME with an email address. MEs who already had an interest in PAs may have felt more strongly about responding to the survey therefore creating a bias. Researchers recommend that more surveys be distributed to attain a larger sample size for future studies.

Sponsor: *N/A*

1706 *(Poster)*

Author: Jacqueline Beeler
Presenter: Jacqueline Beeler
Department: Physician Assistant Studies
Classification: TCOM MPAS Student

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PHYSICIAN ASSISTANTS' ATTITUDES REGARDING THE ETHICS OF HUMAN EMBRYONIC STEM CELL RESEARCH

Purpose: This study investigated physician assistants' attitudes regarding the ethics of human embryonic stem cell research (ESCR) and whether their attitudes were different due to demographics.

Methods: Six state academy/associations (out of twelve selected using a randomized digits table) gave permission to survey their membership. They sent two emails containing a hyperlink to an online survey instrument to their membership. The survey consisted of 17 questions adapted from "Values in Conflict: Public Attitudes on Embryonic Stem Cell Research." The response rate was 21.4% with 650 valid surveys. Data was analyzed using SPSS 12 software (independent t-test, ANOVA, Post Hoc, descriptive statistics, Chi-Square test).

Results: Overall, 72.4% of respondents expressed that they were supportive of ESCR (?2=154.36, p=.001). The respondents indicated their opinions would not change if embryonic stem cells (ESC) could be gathered without destroying the embryo (67.7%) (?2=24.94, p<.001). Respondents did not agree that their opinions would change if research proved that the use of ESC was an effective treatment for a serious disease (68.1%) (?2=81.05, p<.001). They disapproved (55.9%) of creating embryos specifically for use in ESCR (?2=84.76, p=.003) but indicated that conducting research was more important than protecting the embryo (54.3%) (?2=326.66, p<.001). Respondents felt that a difference existed between using embryos created specifically for research to conduct ESCR versus using embryos remaining from in vitro fertilization (53.6%) (?2=78.33, p<.001). Consistently throughout the survey, the religious preference that had the lowest approval of ESCR and the highest approval for protection of the embryo was Evangelical/Fundamentalist. Consistently, the political affiliation that had the lowest approval of ESCR and the highest approval for protection of the embryo was Republican. Conversely, Democrat had the highest levels of approval for ESCR and the lowest for protecting the embryo.

Conclusions: Respondents were supportive of ESCR. The demographics that appeared to most often distinguish between the surveyed PAs' attitudes were religious preference and political affiliation. The fact that individual state academies/associations' email databases may not have been current was a limitation. Likewise, the majority of respondents were Caucasian (89.6%). They might have similar cultural views of the embryo and thus similar opinions concerning ESCR. Research representative of all PAs should be conducted.

Sponsor: *N/A*

1707 *(Poster)*

Author: Victor Villarreal Jr.
Presenter: Victor Villarreal Jr.
Department: Biomedical Sciences
Classification: GSBS Student

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QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIPS FOR ANTIBACTERIAL FUSED MANNICH KETONES

Purpose: Academia and industry has long sought a way to reduce the time and money it takes to discover novel therapeutic drugs. Current methods for discovering drugs include screening large libraries of compounds against a particular target, which is expensive as well as time consuming. While this method will not be replaced, it can be made more efficient by finding out how structural changes in the compounds increase or decrease its efficacy and, then, by designing screening libraries accordingly. We explored fused Mannich ketones as a potential new class of antibacterial compounds in this regard.

Methods: Selected fused Mannich ketones were tested for their efficacy against various bacterial strains. QSAR studies were performed based on measured MIC data and electronic, thermodynamic, topological and property-related descriptors obtained by the computer-aided chemistry (CAChe) software of Fujitsu, Inc. (Beaverton, OR). Molecular models were created by the PM3 semi-empirical quantum chemical method.

Results: We observed a correlation (R =0.7, n = 19) between the compounds' efficacy to inhibit the growth of E. coli and two molecular parameters (polarizability and Max Q+). Another strain (B. subtilis) yielded a weak correlation (R = 0.37, n = 19). However, we have not been able to obtain meaningful QSAR for other bacterial strains.

Conclusions: Using a diverse library of compounds and generating data against a target, one could home in on the functional class of compounds with the highest potency and, then, fine-tune it to increase efficacy. Our preliminary effort to obtain QSAR for antibacterial fused Mannich ketones has been encouraging and prompts an expanded study.

Sponsor: *N/A*

1708 *(Poster)*

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Presenter: Neeraj Agarwal

Department: Biomedical Sciences

Classification: Faculty

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OFFICE OF POSTDOCTORAL EDUCATION

Conclusions: The University of North Texas Health Science Center at Fort Worth (UNTHSC) has recently set up an office for postdoctoral education. The UNTHSC faculty and administration are ready to assist our Postdoctoral fellows in obtaining the training they need for a career in the exciting field of biomedical research. UNTHSC is dedicated to create a research environment that is stimulating, creative and challenging. Our faculty members have distinguished themselves nationally and internationally for their research programs that utilize state-of-the-art technologies. The institution's centers of research excellence provide leadership in the areas of heart disease, diabetes, cancer, infectious diseases, vision, women's health, physical medicine, health disparities, Alzheimer's disease and aging. UNTHSC regards its post-doctoral fellows as a valuable asset and is committed to offering its Postdoctoral Fellows a healthy research environment, which is intellectually enriching and rewarding. To fulfill these goals, a separate curriculum outside of individual research laboratory has been designed to cater the needs of our postdoctoral fellows. Opportunities are provided for grant writing workshops, career skills training, biomedical ethics, biomedical communications, biostatistics, lab techniques and a number of courses for their career advancement. The Office of Postdoctoral Education serves as a liaison between postdoctoral fellows and the University's administration, addressing their needs and concerns, and providing further career development opportunities. The ultimate goal of the Office of Postdoctoral Education is to provide an excellent scientific and supportive environment to advance its postdoctoral fellows to a successful biomedical research career.

Sponsor: *N/A*

1709 *(Poster)*

Author: Søren Klitgaard

Presenter: Søren Klitgaard

Department: Molecular Biology and Immunology

Classification: GSBS Student

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IMAGING USING LONG WAVELENGTH DEPOLARIZED SCATTERED LIGHT FROM SILVER NANOPARTICLES

Purpose: Silver nano-particles are able to both scatter and absorb light in the visible spectrum. The light scattering properties are highly dependent on particle size and shape. The light scattering of the particles are very efficient, they are chemicalle stable and do not suffer photo-bleaching. The purpose of this study is to investigate the use of nano-sized silver colloids as an alternative to molecular fluorophores for imaging in biological tissues.

Methods: In the studies we synthesized silver particles in a variety of sizes ranging from ~40 to 200 nm on the long axis by reducing silver nitrate with citrate. The size distribution of the particles was characterized using Transmission Electron Microscopy (TEM). From the size distribution, the absorption and scattering properties of the silver particles were simulated and compared with the data obtained by absorption spectroscopy and static light scattering. The depolarisation of scattered incident light (vertically polarized) distinguishing the silver particles from a variety of different biologically relevant matrices was imaged using crossed polarizer conditions combined with a camera.

Results: The silver particles displayed a maximum extinction around 410 nm which decreased to approximately 20% of the peak value at observation wavelengths in the red and near infrared part of the electromagnetic spectrum. The anisotropy displayed a local minimum of 0.57 coinciding with the extinction peak but towards the red and near infrared wavelengths the anisotropy decreased to 0.45. Scattering of incident polarized light was used to visualize the silver particles in solutions of Ludox, haemoglobin and red blood cells

Conclusions: We demonstrate that observing the depolarized scattering from silver particles can be used to distinguish between vessels containing silver particles from other scattering media such as silica colloids, haemoglobin and red blood cells.

Sponsor: *N/A*

1800 *(Poster)*

Author: Eva Peña

Presenter: Eva Peña

Department: Alumni

Classification: Alumni

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PROJECT PATHS: EMPOWERING LATINO YOUTH TO CHOOSE HEALTH AND SCIENCE CAREERS

Purpose: One of the goals of Project PATHS is to increase the representation of Latino students in the health professions. Objectives include increasing the number of Latino students reporting interest in health professions and taking college entrance exams. In order to achieve program goals and objectives, Project PATHS has established a community-campus collaborative partnership between the Dallas Independent School District and the University of North Texas.

Methods: Project PATHS adopted an ecological approach based on the social learning theory. Activities are implemented at three levels: personal, behavioral and environmental by a trained group of Latino college students. A quasi experimental design was used for evaluating the program, including an intervention school and a comparison school. A survey instrument was used for data collection. Analyses included Chi-Square, t-test and descriptive statistics. According to results, more students in the intervention school felt that their school had provided them with college information, were confident or very confident about getting financial support to attend college, and intended to take college entrance exams. Valuable lessons learned related to both partnership development and program implementation will also be presented.

Results: More intervention students agreed that in the last six months faculty and staff had talked to them about health science careers (N=776, p=.008). Regarding the possibility of going to college, more intervention students had made preparations, intended to, or had taken action in the past six months (N= 770, p=.043). More intervention students agreed or strongly agreed that their school had provided them with all the information to understand procedures to continue their college education (N=773, p<.005). More intervention students agreed or strongly agreed that they had received information on college applications and admissions procedures within the last six months (N=774, p=.023). More intervention students were very confident about being able to get the financial support to go to college (N=778, p=.033).

Conclusions: Intervention students scored better in their intention and preparation for going to college. Intervention students scored better in receiving college entrance information and support. Intervention students were more confident about obtaining financial support for going to college. All students perceive a graduate degree as a positive outcome.

Sponsor: *Science Education Partnership Award, NIH*

1801 *(Poster)*

Author: Martin Farias III

Presenter: Martin Farias III

Department: Alumni

Classification: Alumni

Martin Farias III 1,2, Jianfeng Ye 1, and Gail D. Thomas 1 1Dept of Internal Medicine, UT Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390 2Dept of Pharmaceutical Sciences, TX A&M HSC, Irma L. Rangel College of Pharmacy, 1010 W. Ave B, Kingsville, TX 78363

CHRONIC NITRATE TREATMENT INDUCES OXIDATIVE STRESS AND ENHANCES SYMPATHETIC VASOCONSTRICTOR RESPONSIVENESS IN CONTRACTING RAT HINDLIMB

Purpose: Sympathetic vasoconstriction in contracting muscles normally is blunted by locally generated vasoactive substances. We have shown that this attenuation, termed functional sympatholysis, is mediated in part by muscle-derived nitric oxide (NO). Skeletal muscle also produces reactive oxygen species (ROS) such as superoxide (O2–) which can inactivate NO. We therefore hypothesized that excessive ROS production would impair functional sympatholysis.

Methods: We induced oxidative stress in rats by infusing the organic nitrate nitroglycerin (NTG; 12.5 mg/kg/d, sc) for 7 days and measured femoral blood flow at rest and during contraction of the left hindlimb. ROS were detected via dihydroethidium stains of each hindlimb.

Results: At rest, sympathetic nerve stimulation (range, 1 to 5 Hz) evoked similar, graded decreases in femoral vascular conductance (FVC, 30% to -69%) in anesthetized Sham and NTG rats (n=9 each). During hindlimb contraction, these vasoconstrictor responses were markedly attenuated, but the degree of attenuation was significantly less in the NTG (FVC, -17% to -50%) vs Sham (FVC, -1% to -31%) rats. Ethidium fluorescence, reflecting intracellular ROS generation, was increased in muscles of NTG rats. Infusion of the O2– scavenger tempol (0.52 mg/kg/min, iv) normalized functional sympatholysis and reduced muscle ROS in NTG rats.

Conclusions: These data indicate that chronic exposure to NTG induces muscle oxidative stress, which impairs functional sympatholysis by enhancing sympathetic vasoconstrictor responsiveness in the active muscles.

Sponsor: *NIH HL06296.*

1802 *(Poster)*

Author: Leslie Napier

Presenter: Jami Kern

Department: Alumni

Classification: Alumni

Jami R. Kern, Ph.D., Jessie M. Lemp, MS and Leslie D. Napier, Ph.D. Alcon Research, LTD., Fort Worth, TX 76134

CLINICAL EXPERIENCE WITH A NEW MULTI-PURPOSE DISINFECTING SOLUTION

Purpose: Clinically examine a new multi-purpose disinfecting solution (MPDS) with a variety of lens materials in asymptomatic and symptomatic subjects.

Methods: Five randomized, double-masked studies conducted at over 40 sites assessed the clinical performance of a new MPDS and its compatibility with traditional and silicone hydrogel (SiH) soft contact lenses. Regimen 1 (OPTI-FREE® RepleniSH™ MPDS) was compared with Regimens 2 (ReNu® MultiPlus® MPS), 3 (ReNu® with MoistureLoc®) and 4 (Complete® MoisturePLUS™). Investigators assessed corneal staining. Subjects recorded rewetting drop use, as well as subjective comfort and symptoms using visual analog scales and Likert-style questionnaires. At a subset of sites, worn lenses were collected for total residual lens lysozyme and lens wettability analyses.

Results: Residual lysozyme of FDA Group IV lenses was significantly lower for Regimen 1 (564.5 and 677.9 µg) compared with Regimens 2 (1062.8 µg) and 3 (959.2 µg, $p<0.0001$). There was significantly less severe corneal staining with Regimen 1 compared to Regimen 2 in SiH lens wearers ($p=0.0019$) and compared to Regimen 3 in symptomatic group IV lens wearers ($p=0.0038$). Group I and IV soft contact lenses cared for with Regimen 1 (58.0° and 5.8°) had significantly lower contact angles (better wettability) than those using Regimen 2 (76.2° and 90.5°; $p<0.002$). Group IV wettability was also significantly better with Regimen 1 (5.3°) compared to Regimen 3 (91.3°; $p<0.0001$). Rewetting drop use was significantly higher in SiH lens wearers using Regimen 4 compared to Regimen 1 ($p<0.004$). Many indices of comfort were significantly higher for those subjects using Regimen 1, particularly for symptomatic subjects who reported significantly better comfort on all 12 of the Likert statements ($p<0.05$).

Conclusions: A new MPDS was shown to have clinical benefits with traditional and SiH lenses compared to three marketed MPS. Choice of lens care regimen can influence ocular health and patient comfort.

Sponsor: *Alcon Research, Ltd.*

1803 *(Poster)*

Author: Diane Thiboutot

Presenter: Lori Johnson

Department: Alumni

Classification: Alumni

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IS THERE A NEED TO SWITCH RETINOIDS TO ENHANCE RESULTS?

Purpose: Acne vulgaris is a chronic skin disease of the pilosebaceous unit affecting approximately 80% of young adults and adolescents. If not appropriately treated, acne may cause serious physical and emotional scarring and can significantly impact the quality of life of those affected by the disease. Topical retinoids, such as adapalene and tazarotene, are an integral part of acne therapy and are considered appropriate first-line therapy, either alone or in combination with antimicrobials, for all cases of acne with the exception of the most severe. These two agents have similar efficacy profiles, however, adapalene has demonstrated a more favorable tolerability profile than tazarotene. Some physicians have reported a strategy whereby they prescribe adapalene 0.1% gel for the first 6 weeks of treatment and then switch to tazarotene cream treatment in response to patient complaints that results are not being seen fast enough. It was hypothesized, however, that both retinoids have a slower onset of action and that the efficacy seen by patients after the switch would have also been seen if they had remained on the original prescription.

Methods: Patients were randomized to one of three treatment arms. Approximately 100 patients each were treated with adapalene 0.1% gel for 12 weeks, tazarotene 0.1% cream for 12 weeks, or adapalene 0.1% gel for 6 weeks followed by tazarotene 0.1% cream for 6 weeks. Percent reduction in total lesions was the primary endpoint measured at the end of 12 weeks.

Results: Our results showed that all three treatment arms resulted in statistically similar reductions in lesions at the end of 12 weeks. All patients experienced an approximate 2 week period of mild irritation at the beginning of the treatment course. Patients who switched retinoids experienced a second irritation period during the 2 weeks after the switch.

Conclusions: Our results suggest that switching retinoids midway through a treatment course does not enhance outcomes.

Sponsor: *N/A*

1804 *(Poster)*

Author: Rustin Reeves
Presenter: Rustin Reeves
Department: Alumni
Classification: Alumni

Rustin Reeves, Robert Wordinger, Anne-Marie Brun, Gary Scott, Harold Sheedlo. Department of Cell Biology and Genetics, University of North Texas Health Science Center, Fort Worth, TX.

TEACHER WORKSHOP AND STUDENT RESEARCH CAPABILITIES ENHANCED THROUGH DEPARTMENT SUPPORT FOR PROJECT SCORE

Purpose: A major highlight this past year for Project SCORE was the purchase, with departmental funds, of a new light microscope for use in our teacher workshops, summer science camps, and summer young scientists program. In the past, we have had very strong support from both the Graduate School of Biomedical Science and the Department of Cell Biology and Genetics for the National Science Foundation's GK-12 program at UNTHSC. We expressed to our chairman the need to expand the training for FWISD science teachers and students in cellular biology, histology, and tissue culture. Our department chairman, Robert Wordinger, Ph.D., acquired funds to purchase an Olympus BX51 light microscope to be used for these outreach activities, and we have since designed a series of workshops for training teachers in the areas of immunofluorescence and cell staining techniques. The microscope is equipped with an Olympus DP70 digital camera and a reflected fluorescence system for DAPI, fluorescein isothiocyanate (FITC-green), and Texas red fluorescence. Image capturing is processed with a Dell Precision Workstation 370 using the DP controller software and visualized with a 20-inch Dell 1905FP UltraSharp flat panel monitor. The total cost for the system was over $25,000, and the first workshop took place on March 1, 2007. Teachers had the opportunity to label certain cell proteins with fluorescent labels, then take pictures of the labeled cells using the new microscope and camera system, save their images to CDs, and take them back to their classrooms to share with their students. FWISD high school science students used the scope for research purposes in the Young Scientists Program this past summer. Furthermore, we plan to incorporate the microscope into our summer science camp for 9th grade SCORE students. This instrument will allow us to offer science workshops for our classroom teachers in Project SCORE for many years to come, give FWISD students training in immunofluorescent staining techniques, and allow us to recruit additional faculty and staff members from our department to help in these outreach activities.

Methods:

Results:

Conclusions:

Sponsor: *National Science Foundation*

BIOMEDICAL SCIENCES ORAL PRESENTATIONS SESSION A

2:00	PM	**Rohini Dhar** INVOLVEMENT OF P70S6K IN CISPLATIN-MEDIATED CELL DEATH	Abstract# 204
2:20	PM	**Linda Mooberry** REDUCED TOXICITY AND SELECTIVE UPTAKE OF PACLITAXEL VIA ENCAPSULATION IN RECONSTITUTED HIGH DENSITY LIPOPROTEIN	Abstract# 213
2:40	PM	**Christina Pacchia** HIGH FREQUENCY VENTRICULAR ECTOPY CAN INCREASE SYMPATHETIC NEURAL ACTIVITY IN HUMANS	Abstract# 304
3:00	PM	**R. Matthew Brothers** EFFECT OF PRAZOSIN ON THE CONTROL OF THE PERIPHERAL VASCULATURE DURING REST, LOW, MILD, AND HEAVY EXERCISE WORKLOADS	Abstract# 311
3:20	PM	**Sherry Sours-Brothers** TRPC1 IS INVOLVED IN CONTRACTILE FUNCTION OF GLOMERULAR MESANGIAL CELLS	Abstract# 400
3:40	PM	**Karen Meeks** DIFFERENTIAL SECRETION OF IL-12 AND IL-18 BASED ON INTRACELLULAR LOCALIZATION OF LISTERIA MONOCYTOGENES	Abstract# 908
4:00	PM	**Ivan T. Lee** CHARACTERIZATION OF SIGMA-1 RECEPTOR LIGANDS USING AN UNAMBIGUOUS ASSAY	Abstract# 1109
4:20	PM	**Archit Gulati** DEVELOPMENT OF A BIOCHEMICAL ASSAY TO SCREEN FOR COMPOUNDS THAT POTENTIALLY INHIBIT MUSSEL GLUE-PROTEIN ACTIVATION	Abstract# 1700
4:40	PM	**Krystle Maccurdy** RESEQUENCING OF THE HUMAN MITOCHONDRIAL GENOME TO AID IN HUMAN IDENTIFICATION THROUGH SEPARATION OF THE COMMON CAUCASIAN HAPLOTYPE GROUP	Abstract# 1704

BIOMEDICAL SCIENCES ORAL PRESENTATIONS SESSION B

2:00	PM	**Courtney A. Bowles** PROGESTERONE-INDUCED REGULATION OF NEUROTROPHINS IN C6 CELLS	**Abstract# 105**
2:20	PM	**Everett S. Nixon** REGULATION OF CYTOSOLIC CALCIUM LEVELS BY INTRACELLULAR CALCIUM CHANNELS IN ROD BIPOLAR CELLS	**Abstract# 705**
2:40	PM	**Gulab S. Zode** BONE MORPHOGENETIC PROTEIN 4 INHIBITS TGF-B2 SIGNALING IN OPTIC NERVE HEAD ASTROCYTES AND LAMINA CRIBROSA CELLS: EXTRACELLULAR MATRIX MODULATION BY GREMLIN IN GLAUCOMA	**Abstract# 710**
3:00	PM	**Amber J. Ondricek** SIGNALING MECHANISMS OF MITOCHONDRIA ASSOCIATED RAT RETINAL GANGLION CELL DEATH IN OPTIC NEUROPATHIES	**Abstract# 712**
3:20	PM	**Jwalitha Shankardas** SUPPRESSED 14-3-3 SIGMA EXPRESSION EXTENDS THE IN VITRO LIFESPAN OF HUMAN CORNEAL AND CONJUCTIVAL EPITHELIAL CELLS	**Abstract# 715**
3:40	PM	**Sheetal Bodhankar** NOVEL ROLE OF NK CELLS IN THE DEVELOPMENT OF ADAPTIVE IMMUNITY AGAINST MYCOPLASMA RESPIRATOY DISEASE	**Abstract# 907**
4:00	PM	**Scott Duncan** MODEL SYSTEMS OF NEURODEGENERATION EXPRESS PROTEINS INVOLVED IN N-ACYLETHANOLAMINE SIGNALING	**Abstract# 1116**
4:20	PM	**David F. Cummings** MODIFICATION OF A SUBTYPE SELECTIVITY MICRODOMAIN IN THE D2 DOPAMINE RECEPTOR CHANGES THE AFFINITY AND FUNCTION OF L750,667	**Abstract# 1602**
4:40	PM	**Diana C. Canseco** SYNTHESIS OF NOVEL SUBSTRATES AS POSSIBLE SEROTONIN RECEPTOR LIGANDS	**Abstract# 1603**
5:00	PM	**Lorie A. Gonzalez** A MECHANISTIC STUDY OF CARISOPRODOL ACTIONS AT $GABA_A$ RECEPTORS	**Abstract# 1604**

PUBLIC HEALTH ORAL PRESENTATIONS

2:00 PM **Theresa D. Quiroz** Abstract# 1501
TO THE BITTER END: DISPARITY ISSUES IN END OF LIFE CARE IN THE UNITED STATES

2:20 PM **Nykiconia Preacely** Abstract# 1506
RACIAL/ETHNIC HEALTH DISPARITIES IN UNITED STATES POULTRY WORKERS

2:40 PM **Harrison T. Ndetan** Abstract# 1512
HEALTH OUTCOME ASSOCIATED WITH THE EXPOSURES OF LEARNING THERAPEUTIC MANIPULATIONS

3:00 PM **Ana Luz Chiapa** Abstract# 1518
DIFFERENCES IN PREGNANCY INTENTIONS AND BIRTH OUTCOMES: A COMPARISON OF BLACK, HISPANIC, AND WHITE WOMEN

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