12TH ANNUAL

RESEARCH APPRECIATION DAY

APRIL 2, 2004

UNIVERSITY *of* NORTH TEXAS HEALTH SCIENCE CENTER

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AGENDA

7:30 - 8:00 AM	Assemble Posters	Atrium/Everett Hall
8:00 - 9:30 AM	Faculty/Non-Student Poster Session	Atrium/Everett Hall
9:30 AM - 12:00 PM	**STUDENT/POSTDOCTORAL/RESIDENT POSTER COMPETITION**	
	Biomedical Sciences	Atrium/Everett Hall
	Public Health, Clinical and Education	Atrium/Everett Hall
12:00 - 2:00 PM	**LUNCH AND KEYNOTE ADDRESS**	Luibel Hall

Welcome
Ronald R. Blanck, D.O., President

Overview of RAD 2004 Activities
Thomas Yorio, Ph.D., Dean
Graduate School of Biomedical Sciences

Institutional Research Efforts
Robert W. Gracy, Ph.D., Associate Vice President
for Research and Biotechnology

Introduction of Keynote Speaker
Marc Hahn, D.O., Dean
Texas College of Osteopathic Medicine

"Clinical Research: A View from the Trenches"
David I. Kaufman, D.O., Professor and Chair
Department of Neurology and Ophthalmology
College of Osteopathic Medicine and College of Human Medicine
Michigan State University

2:00 - 5:00 PM	**STUDENT ORAL COMPETITION**	
	Biomedical Sciences, Session I	Beyer Hall
	Biomedical Sciences, Session II	Mini Auditorium
	Public Health, Clinical and Education	EAD-506
ALL DAY	**VENDOR FAIR**	Atrium/Everett Hall
5:00 - 5:30 PM	Remove Posters	
5:30 PM	**AWARD CEREMONY**	Luibel Hall

KEYNOTE SPEAKER

David I. Kaufman, D.O.
Professor and Chair
Department of Neurology and Ophthalmology
College Osteopathic Medicine and College of Human Medicine
Michigan State University

"Clinical Research: A View from the Trenches"

David I. Kaufman, D.O., serves as professor and chair of the department of neurology and ophthalmology for the College of Osteopathic Medicine and College of Human Medicine at Michigan State University.

He also directs the Neurology Osteopathic Residency Organization and the Consortium of Osteopathic Residencies in Ophthalmology for the university. In addition to his work at MSU,
Dr. Kaufman also serves as the medical director of neurology for Sparrow Health Systems in Lansing, Mich.

Dr. Kaufman is frequently recognized for both teaching and patient care. His students have chosen him numerous times to receive the Distinguished Lecturer Award, the Hooding Award and the Golden Apple Teaching Award. He received the American Academy of Ophthalmology Achievement Award in 2002 and is the Louisa Burns Memorial Lecturer for 2003. Dr. Kaufman is also listed in 2004's Best Doctors in America, a distinction he's held since 1996.

His research interests include using the visual system to assess prognosis and treatment strategies for brain diseases such as multiple sclerosis and stroke; investigating clinical neuro-visual disorders, their prognoses and treatment strategies; applying pattern-electoretinogram, visual evoked potentials and contrast sensitivity in neuro-ophthalmology disease; analyzing neuro-transmitter and neurological disease using retinal-cortical time; and researching magnetic resonance imaging and neuro-ophthalmology.

He received his medical degree from Philadelphia College of Osteopathic Medicine and earned his bachelor of science degree from the University of Wisconsin, where he also completed a residency in neurology. Dr. Kaufman did a fellowship in neurophysiology at UW, and he was a Harvard Research Fellow in clinical neuro-ophthalmology at Massachusetts General Hospital in Boston.

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 11,000 individuals around the world. Total sales for 2003 exceeded $3.4 billion, with activity in more than 170 markets. One of the cornerstones of Alcon's success is the company's commitment to Research and Development. Housed at the company's headquarters in Fort Worth is the 550,000 square-foot William C. Conner Research Center, the largest and most sophisticated eye research center in the world. Over the next four years, Alcon plans to spend nearly $1.5 billion on eye-related research, 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 Session I of the 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 Oral Presentation Awards are given to the top two basic sciences student oral presentations in Session II of the two oral presentations. The GSA Poster Presentation Awards are given to the top five student poster presentations 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 organization that serves as a forum for student concerns and activities. The purpose of the PHSA is to facilitate student-student and student-faculty communication and cohesiveness within the School of Public Health. The organization works on issues pertaining to curriculum revision, research opportunities, student participation, and alumni fellowship through social and professional activities.

The objectives of PHSA are: 1) to provide members with resources that will enhance their educational careers; 2) to foster communication among students, SPH faculty, staff, and administration; 3) to promote research opportunities through collaborative public health approaches to disease prevention and health promotion; 4) to foster a prosperous graduate school experience for its members; and 5) to encourage fellowship among alumni, SPH students, faculty, and staff.

The Public Health Student Association sponsors Research Appreciation Day student awards for the top two oral presentations and the top two poster presentations as determined 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 other health professionals 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.

PROFESSIONAL AND CONTINUING EDUCATION AWARDS

Professional and Continuing Education (PACE) is pleased to announce the availability of two new research awards for outstanding poster presentations at the University of North Texas Health Science Center Annual Research Appreciation Day. 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, administrators and certified health education specialists. PACE registers more than 10,000 healthcare professionals at more than 500 activities each year in 43 states.

The PACE Pre-doctoral Research Award and the PACE Post-doctoral Research Award 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.

The Research Appreciation Day 2004 PACE Award judges are:

Dr. John Fling
Acting Chair, Associate Professor Pediatrics
UNT Health Science Center

Dr. Gregory McQueen
Senior Vice President for Academic Affairs
UNT Health Science Center

Dr. Bobby Jones
Manager, Division of Epidemiology & Health Information
Tarrant County Public Health Department

TECH FORT WORTH INNOVATION AWARD

The Tech Fort Worth Innovation Award is sponsored by Tech Fort Worth, a privately funded non-profit business incubator designed to provide specialized and industry-specific business assistance to technology start-up companies. This economic development effort provides a mechanism that facilitates the growth and development of emerging technology companies in Fort Worth.

Its mission is to encourage business development in the Greater Fort Worth area by attracting, growing, and graduating successful technology companies that become financially viable and freestanding.

The Incubator invests time and expertise in emerging companies and entrepreneurs that demonstrate the potential for economic and commercial success. Technology companies such as these also diversify the Fort Worth economy and make it less reliant on a single industry, while creating high-wage and high-quality jobs.

To increase the probability of success by the portfolio companies, ensure a high graduation rate, and sound decision making by the entrepreneurs, Tech Fort Worth provides a wide range of specialized business services that, in a pro-active approach, are critical for the participating companies.

In addition, Tech Fort Worth offers introductions and connections to a network of corporate investors, such as venture capitalists, investment and merchant bankers, angel networks and matchmaking services. Also, Tech Fort Worth manages a new, 20, 000 sq. feet facility that offers executive suites, internet access, conference rooms, ample parking, and 24 hour security to client companies.

See Tech Fort Worth online at www.techfortworth.org

Tech Fort Worth
1150 S. Freeway, Suite 129
Fort Worth, Texas 76104
817.339.8968
817.332-6465 (F)
info@techfortworth.org

The Research Appreciation Day 2004 Innovation Award judges are:

Clyde Higgs
Executive Director
Tech Fort Worth

Robert McClain, Ph.D.
Director, Technology Development & Commercialization
UNT Health Science Center

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 2004 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.

GRADUATE SCHOOL OF BIOMEDICAL SCIENCES JUDGES

The Research Appreciation Day 2004 student poster presentation judges are:

Craig Burnside, Ph.D.
Texas Wesleyan University

Oswald Dauvergne, Ph.D.
Southern University

Edward Elko, Ph.D. (Head Poster Judge)
Emeritus, UNT Health Science Center

Debra Fleenor, Ph.D.
Alcon Research, Ltd.

William Garner, Ph.D.
Retired, Alcon Research, Ltd.

Nasreen Jacobson, Ph.D.
Alcon Research, Ltd.

Michael Lawrence, Ph.D.
UT Southwestern Medical Center

Kerry Markwardt, Ph.D.
Alcon Research, Ltd.

Leslie Napier, Ph.D.
Alcon Research, Ltd.

Allan Shepard, Ph.D.
Alcon Research, Ltd.

Roberta Troy, Ph.D.
Tuskegee University

The Research Appreciation Day 2004 postdoctoral fellows poster presentation judges are:

Gregory Buck, Ph.D.
Texas A&M University - Corpus Christi

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

Terry Wiernas, Ph.D.
Alcon Research, Ltd.

The Research Appreciation Day 2004 student oral presentation judges are:

Annita Bens, Ph.D.
MedTrials, Inc.

Robert Collier, Ph.D.
Alcon Research, Ltd.

Patrick Cooke, Ph.D.
Affymetrix, Inc.University

Julie Crider, Ph.D.
Alcon Research, Ltd.

Jami Kern, Ph.D.
Alcon Research, Ltd.

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

Michael Rudick, Ph.D.
Emeritus, Texas Woman's University

SCHOOL OF PUBLIC HEALTH JUDGES

The Research Appreciation Day 2004 poster and oral presentation judges are:

Marcus Martin, M.A., M.P.H., Ph.D.
Director of Research
Foundation for Community Empowerment

Susie R. Mikler, M.P.H., Ph.D.
Faculty Associate
UT Houston School of Public Health
UT Southwestern Medical Center

Amy Raines, M.P.H.
Team Leader
City of Fort Worth Public Health Department

TEXAS COLLEGE OF OSTEOPATHIC MEDICINE JUDGES

The Research Appreciation Day 2004 poster presentation judges are:

Rhett Fredric, M.D.
Private Practice

Mark Laney, M.D.
Cook Children's Network

Thomas O'Shea, D.O.
Private Practice

Wayne Williams, M.D.
JPS Health Center - South Campus

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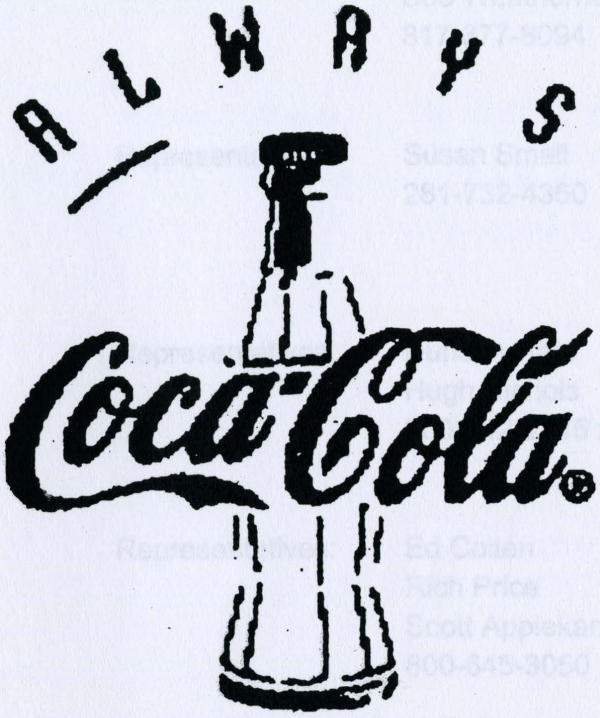

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100

Author: Janice Knebl **Presentor:** Janice Knebl
Department: INTERNAL MEDICINE
Classification: Faculty (Not for Competition)
Janice Knebl, David Orr, Walter McConathy, Peter Lam, Kevin Crutchfield
Internal Medicine, University of North Texas Health Science Center, Fort Worth, TX and
Cerebrovascular Medicine, New Health Science, Inc., Rockville, MD, USA

INTRACRANIAL HEMODYNAMICS IN ALZHEIMER'S DISEASE

Purpose: To determine the hemodynamic characteristics of Alzheimer's Disease through the utilization of Dynamic Cerebrovascular Assessment (DCA) Methodology. Dementia of the Alzheimer's Type is associated with the deposition of beta-amyloid protein in the vascular endothelium and parenchyma of the brain. Global low cerebral blood flow and hypometabolism have been described, however, hemodynamics associated with these changes have not been elucidated. Utilization of Transcranial Doppler Ultrasound along with DCA Methodology to evaluate intracranial hemodynamics has not been studied in Alzheimer's Disease

Methods: Patients (n=56) with a diagnosis of probable dementia of the Alzheimer's type, and 31 age-matched controls, voluntarily underwent full Transcranial Doppler (TCD) evaluation with Dynamic Vascular Analysis (DVA) to characterize the hemodynamics of the intracranial vasculature.

Results: The DVA velocity-impedance ratio, a surrogate marker for relative blood flow, was significantly diminished in the following TCD locations:M1, A1, C1, C2, C4, VA, BA, P1, and P2. These values also correlated with the Folstein mini-mental status scores. Pulsatility Index was significantly increased in these TCD locations: M1, A1, C1, C2, C4, VA, BA, P1, and P2. TCD sites representing extracranial blood flow, such as the ophthalmic artery, did not differ between Alzheimer's subjects and controls. In addition, the systolic upstroke acceleration did not differ between the two groups, suggesting that the global low flow is not secondary to diminished proximal flow. In fact, the DVA index relating acceleration to velocity was increased in the Alzheimer's subjects suggesting increased kinetic expenditure to maintain forward flow.

Conclusions: Alzheimer's Type Dementia appears to have characteristic cerebral blood flow hemodynamics. The increased impedances dramatically alters forward force and ultimately cerebral blood flow. Based on this data it is possible that low cerebral blood flow in Alzheimer's disease is secondary to increased impedance in the small capacitance vessels, the arterioles. This may be due to increased amyloid deposition with alteration of vascular architecture, increased intracranial pressure (explaining the unaltered extracranial vessel hemodynamics), or a combination of both.

Sponsor:

101

Author: Douglas Mains **Presentor:** Douglas Mains
Department: Health Management & Policy
Classification: Faculty (Not for Competition)
Douglas A. Mains, DrPH
Thomas J. Fairchild, PhD
Health Management and Policy
Special Projects on Aging
School of Public Health
Fort Worth, TX 76107

COMPREHENSIVE CAREGIVER CHOICES PROGRAM

Purpose: The primary goal of the Comprehensive Caregiver Choices (C3) Program was to provide information, referral and educational services for HMFW employees using a concierge model to assist those who cared for or assisted with the care of elderly family or friends outside of the workplace.

Methods: The C3 Program utilized an MSW and other educators to provide services to employee caregivers, including educational sessions, information and referral, and one-to-one counseling. Evaluation of the C3 Program included process indicators (number of contacts, informational presentations, information requests, etc.), participant reaction to educational sessions, and follow-up surveys of program participants.

Results: Overall responses show that educational seminar participants indicated a knowledge gain of 1.8 points in pre/post session knowledge on a 5-point scale. Overall rating of the educational sessions averaged 4. on a 5-point scale and average ratings of objective attainment and instructors ranged from 4.6 to 4.9 on a 5-point scale. Preliminary examination of the follow-up surveys indicates the average response regarding the usefulness of services is 4.5 on a 5-point scale, with 98% rating the services as helpful or very helpful. Eight-nine percent of the respondents made contact with services they were referred to and 85% found the information to be useful. The employees surveyed thus far reported an average stress level of 1.65 prior to utilizing C3 Program services and post-service stress level of 4.2, with an average increase of 2.5 (on a 5-point scale with 1=very stressed and 5 = very calm). Quality of life improvement averages 4.6 on a 5-point scale, with 67% rating their quality of life as greatly improved.

Conclusions: The concierge model works well to the extent that employee caregivers are willing to self-identify and seek services or attend educational seminars. The C3 Program served approximately 3% of the total workforce at Harris Methodist. It is believed that continued outreach combined with increased access to caregiver information and scheduling of educational seminars to accommodate workers on various shifts will increase the participation rate.

Sponsor: *United Way of Metropolitan Tarrant County*

102

Author: Hong Guo **Presentor:** Hong Guo
Department: INTEGRATIVE PHYSIOLOGY
Classification: Postdoctoral Fellow/Resident
Hong Guo1, Frederic Schaller2, Scott A. Smith3, Nancy Tierney2, K Vesenius2, and Xiangrong Shi1
1)Department of Integrative Physiology and 2)Department of Internal Medicine,
University of North Texas Health Science Center, Fort Worth, Texas 76107 3)Department of Health Care Sciences, University of Texas Southwestern Medical Center,
Dallas, Texas 75390

CARDIOVASCULAR FUNCTION WITH AGING - TRANSFER FUNCTION ANALYSIS

Purpose: This study was designed to test the hypotheses that cardiovascular variability between cardiac output (CO) and mean arterial pressure (MAP) and between MAP and heart rate (HR) signals when assessed using transfer function analysis was altered with age.

Methods: Six-min HR, CO (thoracic impedance), tonometer systemic arterial pressure (SAP), systemic oxygen saturation (SO2S, Oximeter), and regional cerebral oxygen saturation (RO2S, near-infrared spectroscopy) were continuously recorded (at 400 Hz) from 8 elderly (71±2 yr) and 7 young (27±2 yr) healthy volunteers during supine resting and graded lower body negative pressure (LBNP), respectively. These cardiovascular data were then selected, resampled, and converted using fast Fourier transform. The ratio of cross-spectrum to auto-spectra in low-frequency (LF, 0.04-0.14 Hz) and high-frequency (HF, 0.15-0.30 Hz) between two signals (CO-MAP and MAP-HR) was computed for transfer function analysis using Welch Spectral estimator.

Results: LF CO-MAP coherence was not different between the groups, whereas HF CO-MAP coherence was consistently (P=0.04) greater in young than elderly group. However, CO-MAP coherence in both elderly and young groups was <0.5 and CO-MAP phase was not statistically different from 0 in LF or HF. MAP-HR phase was all located in positive phase (>0 degree) in both groups, suggesting that MAP variability preceded HR variability. MAP-HR coherence was >0.5 in young and <0.5 in elderly group at both LF and HF spectra (group difference P=0.01). Overall MAP-HR gain was significantly smaller in the elderly. However, in terms of unit decrease in CO or stroke volume (SV), increase in total peripheral resistance (TPR) was significantly greater in elderly (-14.14+/-3.03 PRU/L/min or -0.64+/-0.09 PRU/mL) than young (-4.84+/-0.43 PRN/L/min or -0.24+/-0.01 PRU/mL) group. LBNP did not change SO2S, but decreased RO2S, suggesting cerebral under-perfusion. Aging appeared to be associated with a greater decrease in RO2S in terms of unit decrease in CO or SV (elderly vs young: 5.71+/-0.75 vs 3.04+/-0.14 %/L/min or 0.25+/-0.04 vs 0.15+/-0.01 %/mL).

Conclusions: We concluded that CO-MAP oscillations were not significantly synchronized or not related in a simply linear fashion in both elderly and young subjects. Aging not only diminished MAP-HR transfer function gain, but also weakened MAP-HR coherence. A greater drop of RO2S during central hypovolemia in the elderly appeared to explain orthostatic intolerance associated with aging.

Sponsor: *NIH Grant HL65613*

103

Author: Laurel Blackman **Presentor:** Laurel Blackman
Department: INTERNAL MEDICINE
Classification: TCOM Student
Laurel Blackman, MSIII, Walter McConathy, Ph.D., and Janice Knebl, DO, MBA, Department of Internal Medicine, TCOM, UNTHSC, Fort Worth, Texas, 76107.

ATTITUDES, BELIEFS AND PERCEPTIONS ABOUT GERIATRICS BETWEEN MEMBERS OF STUDENT CHAPTER OF THE AMERICAN GERIATRICS SOCIETY MEMBERS VERSUS NON-MEMBER PEERS.

Purpose: Older adults represent an expanding segment of our population, with those greater than 85 years making up the fastest growing subset. The UNTHSC/TCOM Student Chapter of the American Geriatrics Society (SCAGS) offers its members a variety of extracurricular experiences and opportunities designed to spotlight trends shaping the specialty field of geriatrics and impacting care of older adults. This study assessed differences in attitudes between SCAGS members exposed to club programming versus their non-member peers to determine whether SCAGS membership stimulated a cadre of medical students with significant interest in geriatrics.

Methods: First and second year medical students completed a survey questionnaire modified from one previously reported (Alford et. al., J Am Geriatric Soc. 49: 782, 2001). This survey was designed to be completed in five minutes and was given at the end of the academic year. Scale for attitude responses ranged from 1 (strongly disagree) to 6 (strongly agree). Results of the questionnaires were analyzed for differences between SCAGS members (n=68) and nonmembers (n=160) in individual responses and responses grouped into three domains. Answers to non-scaled questions regarding participation and programs for both groups were also tallied.

Results: Comparing SCAGS member and nonmember attitudes revealed 8 of 23 (35%) responses were different ($p<0.05$). When grouped into the three domains, no differences were noted in attitudes between the two groups ($p>0.4$). However, 4 of 7 (57%) responses regarding aging processes were significantly different while the attitudes regarding careers (1 of 5, 20%) and providing medical care (3 of 11, 27%) showed less differences. SCAGS members citied events with actual geriatric clinic patients as activities that most prepared them for caring for older patients. Non-member students responded with club dues and no need for learning about geriatrics as reasons for not joining SCAGS.

Conclusions: SCAGS members possess attitudes that are positively related to the field of geriatrics. Understanding opinion differences between students exposed to SCAGS programming versus their non-member classmates offers an opportunity to make important contributions in furthering efforts to design, fund and implement geriatrics curricula as well as attract, foster and prepare future geriatric-friendly physicians and specialty-trained geriatricians. This study points to the continued need for faculty, community and financial investment in SCAGS efforts.

Sponsor:

104

Author: Martine Pastorcic **Presentor:** Martine Pastorcic
Department: PHARMACOLOGY & NEUROSCIENCE
Classification: Postdoctoral Fellow/Resident (Not for Competition)
Martine G. Pastorcic and Hriday K. Das
Department of Pharmacology & Neuroscience
UNT Health Science Center at Fort Worth
Fort Worth, TX 76123

CELL-SPECIFIC REGULATION OF THE PRESENILIN-1 GENE

Purpose: Presenilin-1 (PS1) gene regulation plays a critical role to control Alzheimer□s disease (AD) and mammalian development. Therefore, the major purpose of this study is to determine if the PS1 promoter is utilized in alternative ways in SK-N-SH and SH-SY5Y neuronal cell lines resulting in the cell-specific expression of the PS1 gene.

Methods:
A promoter region between 118 and +178 has been mapped in SK-N-SH and SH-SY5Y cells. 5□ and 3□ deletion analysis followed by transfection of PS1-promoter/CAT constructs into these cell lines were used to show that sequences both upstream and downstream of +1 site play a crucial role for PS1 transcription in both these cells. Site directed mutagenesis revealed that Ets factor binding sites at 10 and +90 are important for PS1 expression. Antibody supershift assay and the yeast one hybrid screening confirmed the binding of Ets factors at 10 and +90 promoter regions.

Results: Either upstream or downstream sequences are sufficient to direct transcription in SH-SY5Y cells, whereas in SK-N-SH cells 5□-deletions past the +1 site eliminate over 95% of transcription. To understand how the promoter may be utilized alternatively in different cell types we have examined the effect of point mutations in Ets elements. Altering an Ets motif at 10 eliminates 80% of transcription in SK-N-SH cells whereas the same mutation has only a minor effect in SH-SY5Y cells. Conversely, the Ets element at +90, which eliminates 70% of transcription in SH-SY5Y cells has a lesser effect in SK-N-SH cells. In both cell types a promoter including mutations at both 10 and +90 Ets sites looses over 90% transcription activity indicating the crucial importance of these two Ets motifs. Hence the differential expression in each cell type may be at least partially determined by Ets factors and the 10/+90 sites. We show here that ERM recognizes specifically Ets motifs on the PS1 promoter located at 10, +90, +129 and +165 and activates PS1 transcription.

Conclusions: Cell-specific expression of the PS1 gene was studied using SK-N-SH neuronal cell lines. Elimination of +1 site resulted in complete ablation of PS1 expression in SK-N-SH cells. In SH-SY5Y cells alternative initiation mechanisms were clearly indicated by significant level of promoter activity conferred by either of the two PS1 fragments (-118 to +6) or (+2 to +178). Ets transcription factors binding to 10 and +90 promoter regions appeared to be critical for the expression of the PS1 gene in both these cells.

Sponsor: *NIA/NIH*

105

Author: Ritu Shetty **Presentor:** Ritu Shetty
Department: PHARMACOLOGY & NEUROSCIENCE
Classification: GSBS Student
*1) Ritu A. Shetty**
*2) Nathalie Sumien**
3) Rajindar Sohal+
*4) Michael J.Forster**
**- UNTHSC at FortWorth, Fortworth TX-76107.*
+- Univ. Of Southern California, Los Angeles, CA- 90033.

SHORT-TERM COENZYME Q10 SUPPLEMENTATION ENHANCES COGNITIVE PERFORMANCE IN AGING MICE

Purpose: Brain aging is accompanied by an increase in oxidative stress and mitochondrial dysfunction. Coenzyme Q (CoQ) is an important component in the electron transport chain and is thought to serve as a potent antioxidant. It is also known that CoQ content declines with aging and the levels can be restored in older mice by supplementation. However the effects of CoQ10 supplementation on behavior are not known. Therefore the purpose of this study was to determine the short-term effects of CoQ10 supplementation on psychomotor and cognitive performance in young and old mice.

Methods: Separate groups of young (4 months) and old mice (18 months) were fed a control diet or a diet supplemented with low or high concentrations of CoQ10 for a period of 15 weeks. The low and high supplemented diets yielded a daily CoQ10 intake of approximately 148 or 654 mg/kg, respectively. After 6-weeks on the diets the mice were subjected to a battery of age-sensitive behavioral tests for cognitive and psychomotor performance.These tests include locomotor activity, coordinated running performance, swim maze and startle response.

Results: In the swim maze task, CoQ10-treated mice tended to exhibit faster learning of a swim maze task, an aging-sensitive measure of cognitive performance. Diet supplementation with CoQ10 also improved the ability of the old mice to retain information in swim maze task, an effect that was not evident in the younger mice. In tests for auditory and shock startle reflex and coordinated running ability, CoQ10 supplementation failed to improve performance.

Conclusions: Results suggest that short-term CoQ10 supplementation improves impaired cognitive function in older mice, but fails to ameliorate age-impaired psychomotor function.

Sponsor:

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ESTROGENS PROTECT HUMAN SK-N-SH NEUROBLASTOMA CELLS AGAINST H2O2 TOXICITY

Purpose: Estrogens are known as potent neuroprotective agents in a variety of animal and cell culture models. Here, we assessed the protection of 17beta-estradiol (17beta-E2) against H2O2 toxicity in human SK-N-SH neuroblastoma cells and the mechanisms involved in estrogen□s protection. To further address the requirement of estrogen receptors (ERs) in estrogen enhancement of neuronal survival, we tested the effects of 17alpha-estradiol (17alpha-E2), a weak estrogen, and Ent-estradiol (Ent-E2), the complete enantiomer of 17beta-E2.

Methods: We studied neuroprotection of estrogens against H2O2 in cell viability using calcein AM assay. We further investigated the mechanisms involved in estrogens' protection, we measured caspase-3 activity and mitochondrial membrane potential. We also monitored intracellular calcium hemeostasis by fura-2 and reactive oxygen species (ROS) by DCF.

Results: All three estrogens, 17beta-, 17alpha- and ent-E2 attenuated H2O2 (100microM and 150microM)-induced cell death. With 30 min treatment, H2O2 increases intracellular calcium ([Ca]i) more than 5-fold at the concentration of 150mM. With 2h pretreatment, 0.1 microM 17beta-E2 significantly reduced this H2O2-induced intracellular calcium increase by 50% and 1 microM 17beta-E2 decreased [Ca]i increase by about 65%. 17alpha- and Ent-E2 showed similar efficacy in reducing H2O2-induced [Ca]i at the concentration of 1 microM. Interestingly, ICI182,780, an ER antagonist, did not block 17beta-E2□s effects, but also showed attenuation of H2O2-induced [Ca]i increase at 300 nM. All three estrogens (17beta-, 17alpha- and Ent-E2) attenuated H2O2-induced caspase-3 activation and this effect could not be blocked by ICI182,780. To investigate the acute effects of estrogens on mitochondrial membrane potential, we treated SK-N-SH cells with 3.0 mM H2O2 to induced mitochondrial potential collapse, 17beta-E2 significantly protected mitochondria at both 0.1 microM and 1 microM. 17beta-E2 did not abate intracellular ROS accumulation as prompted by H2O2 exposure, and indicated that 17beta-E2 did not act as ROS scavenger.

Conclusions: 17beta-E2 and non-feminizing estrogens, 17alpha- and ent-E2, protected SK-N-SH cells against H2O2-induced toxicity by maintaining intracellular calcium homeostasis, stablizing mitochondria and subsequently inhibiting apoptosis cascades. Our data suggest classical estrogen receptor-medicated effects are not involved in the protection.

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17 B-ESTRADIOL ATTENUATES BLOOD BRAIN BARRIER DISRUPTION THROUGH DOWN-REGULATION OF MATRIX METALLOPROTEINASES AFTER CEREBRAL ISCHEMIA-REPERFUSION INJURY IN FEMALE RATS

Purpose: Changes in blood-brain barrier (BBB) integrity have been described in several neurological disorders including stroke. Disruption of the BBB is associated with alterations in brain function as well as brain edema and neuronal death. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that can degrade extracellular matrix causing disruption of BBB integrity, amplify inflammatory infiltrates, demyelinate neurons and cause cell death. MMPs are up-regulated after clinical and experimental cerebral ischemia-reperfusion injury. Estrogen and related compounds have been show to be potently neuroprotective against stroke. We hypothesize that 17b-estradiol (E2) attenuates BBB disruption after transient middle cerebral artery occlusion through down-regulation of MMPs expression.

Methods: Ischemia-reperfusion injury was induced by temporary middle cerebral artery occlusion (MCAO) for 1 hr. E2 (100mg/kg) or vehicle was administered s.c. 2hr before MCAO in ovariectomized rats. Cortex and subcortex tissue was harvest in E2 or vehicle treated animals at 2hr, 4hr, or 24hr of reperfusion. MMP-2 and MMP-9 protein expression was determined by western blot. In another group of experiments, vascular permeability was quantitatively evaluated by fluorescent detection of extravasated Evans blue dye at 4hr of reperfusion in E2 and vehicle treated rats. MMP activity assays were performed using a fluorogenic substrate TNO211.

Results: Western blot showed that MMP-2 and MMP-9 protein expression was elevated as early as 2 hr and peaked at 4 hr of reperfusion in the ischemic cortex. In rats treated with E2, MMP-2 and MMP-9 were markedly reduced in the ischemic cortex. BBB disruption as measured by Evans Blue Dye extravasation was significantly increased in the ischemic cortex and subcortex. E2 prevented more than 50% of BBB disruption in the ischemic cortex and 30% in the ischemic subcortex at 4hr of reperfusion. And MMP activity was significantly decreased by E2 at 4 hours reperfusion.

Conclusions: Our data suggest that E2 is neuroprotective and can attenuate BBB disruption in part by down-regulation of MMP expression after cerebral ischemia-reperfusion injury. (Supported by NIH grants AG10485 and AG 22550 and Mitokor, Inc)

Sponsor: *NIH AG10485*

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HABITUATION OF THE STARTLE REFLEX AS A NOVEL MEMORY TEST IN MICE.

Purpose: Repeated exposure to the same stimulus often results in a weakening of the response. This process is known as habituation and represents a basic form of memory. The purpose of the present study was to determine the utility of a novel behavioral paradigm in measuring the stability of a habituated response in the acoustic startle reflex test.

Methods: Ten young adult male C57B6/JNia (B6) mice were tested for 5 consecutive days and then tested again after a 1-week retention interval. In each test, a mouse was individually placed in an acrylic cylinder that was positioned on top of a movement-sensitive platform and subjected to a series of 30 trials in which brief noise bursts were sounded.

Results: The amplitude of an animal's startle reflex to the noise generally decreased from the first trial to the last trial of a session, demonstrating within-session habituation. Additionally, first-trial amplitudes tended to decrease from one session to the next, demonstrating between-session habituation. Importantly, first trial amplitude in the 1-week retention test remained low, demonstrating long-term habituation of a response. However because B6 mice suffer significant hearing loss as they age, a modification of the startle paradigm was devised to detect habituation that was not dependent upon hearing ability. The same 10 mice were placed in the startle chamber and exposed to a series of 20 foot shocks. Mice were tested for 5 consecutive days and tested again after a 1-week retention interval. Although the startle response to the shock stimulus tended to decline both within each session and across sessions, these declines were not significant.

Conclusions: Therefore, the current acoustic startle paradigm may be able to detect differences in cognitive abilities among B6 mice of the same age group, but not between young and old mice. In future studies, a greater number of shock trials within a session may yield a more conclusive demonstration of between session habituation in the shock startle paradigm.

Sponsor:

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QUANTITATIVE EVALUATION OF OXIDIZED FIBRINOGEN FOR THE EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

Purpose: Oxidative damage is central to the pathogenesis of neurodegenerative disorders including Alzheimer□s disease (AD). Specific oxidation-sensitive proteins (OSPs), when oxidatively modified, initiate a cascade leading to apoptosis and the clinical/behavioral symptoms of the disease. We have utilized proteomic methods to locate and identify the OSPs characteristic of AD. The goals of these studies are: (a) to gain insight into the mechanism of the disease and (b) to use these OSPs as biomarkers for the early diagnosis and monitoring of the progression of the disease.

Methods: Plasma from individuals with AD (and age-matched controls) was obtained, and the OSPs were resolved by 2D-PAGE and located by immunostaining for oxidized proteins. The OSPs were recovered and identified by mass spectroscopy.

Results: In the plasma two predominant OSPs were identified as oxidized isoforms of alpha-1-antitrypsin and the gamma chain of fibrinogen. The evaluation of levels of these oxidized proteins is being performed by an ELISA-based immunoassay. Dinitrophenyl hydrazine (DNPH) is used to derivatize oxidized proteins, and the removal of excess DNPH from a sample is critical to avoid false-positives. To date there has not been a known method for removal of the underivatized DNPH and thus no ELISA-based immunoassay for individual oxidized proteins.

Conclusions: We now report a method for removal of DNPH, which permits rapid, quantitative screening for oxidized fibrinogen and total fibrinogen. This method is being optimized and evaluated as an early diagnostic screen for individuals who are "at risk" for the disease. Thus, these oxidized protein isoforms may provide important information on the mechanism and aid in early diagnosis of Alzheimer□s disease.

Sponsor: *ATP*

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CHARACTERIZATION OF ANTIOXIDANT ACTIVITIES OF TAMARIND SEED HUSKS

Purpose: Botanicals are being found to contain extremely potent antioxidants. For example, polyphenols, including phenolic acids, flavonoids and condensed tannins contain multiple hydroxyls, which serve as traps for reactive oxygen species (ROS) and reactive nitrogen species (RNS). Recently, our laboratories have examined the effects of such flavones from grape seed extracts on neuronal cells and found that they prevent oxidation-induced cell death by increasing Bcl-2 and decreasing levels of Bax, which regulate apoptosis. Tamarind (Tamarindus indica) is an evergreen tree of the tropics and its seeds are an abundant source of nutritional phytochemicals. However, the seed husks, which make up 30-40% of the seed, are generally considered waste products of the food industry.

Methods: The antioxidant activity of tamarind seed extracts was evaluated by neutral red dye uptake assays after glucose oxidase induced oxidative damage to an established rat retinal ganglion cell line, RGC-5. We have also characterized acetone extracts of the seed husks by chemical, physical and chromatographic methods.

Results: The extracts were found to scavenge both oxygen-center radicals (ABTS/OxyMetHb assay) and nitrogen-center radicals (DPPH assay). The extract was also effective in biological based assays. For example, it prevented the oxidation-induced hemolysis of both normal and G6PD-deficient erythrocytes. The extracts were also effective in preventing oxidation-induced apoptosis in neuronal cells. At high levels some toxicity was observed, however this compound(s) may be removed by differential solvent extraction or chromatography. Chemical, physical and chromatographic analyses indicate similarity with grape seed oligomeric proanthocyanidins (OPC), with an average degree of polymerization of six. The levels of condensed tannin appear to be higher than in grape seed.

Conclusions: These data suggest that tamarind seed husks have potent antioxidant properties and should further be explored for use as potential antioxidants.

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AN ASSAY TO QUANTIFY AROMATASE ACTIVITY IN MALE AND FEMALE RAT SPINAL CORD

Purpose: The purpose of the present experiment was to develop and optimize an assay for aromatase in the spinal cord that is sensitive, reliable and specific.

Methods: These assays use tritiated water-release (modification of Lephart & Simpson method) wherein the release of tritium from the substrate androstenedione into the aqueous phase is quantified.

Results: In both the male and female rat spinal cords, we found the optimal protein concentrations (90-140 ug/ml in male, 90 ug/ml in female) and the optimal time of incubation (45 min for male, 15 min for female). In addition, the activity of aromatase can be inhibited by 4-hydroxyandrostenedione (an aromatase inhibitor) at concentrations of 2mM for male and 500uM for female.

Conclusions: Thus, we believe the assay can be utilized to investigate changes in aromatase activity in the spinal cord following applications of pain stimuli (i.e., von frey monofilaments) and after pharmacological manipulations (i.e., estrogen implants).

Sponsor:

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PROGESTERONE PROTECTS AGAINST GLUTAMATE TOXICITY IN ORGANOTYPIC EXPLANTS OF THE CEREBRAL CORTEX

Purpose: Women over the age of 65 have a two to three times higher prevalence of Alzheimer□s disease, suggesting that hormone deficits may play a role in the disease causation. In ovariectomized rodents, estrogen replacement has been illustrated to have a beneficial effect in cognitive tests, and was associated with parallel changes in morphological, neurochemical, and molecular deficits also seen in AD. The fact that not just estrogen, but progesterone also declines with age, drives the hypothesis that progesterone my also play an important role in neuroprotection. Previously, our laboratory has illustrated that progesterone can elicit signal transduction pathways known to be associated with neuroprotection. In order to extend this data, we evaluated possible mechanisms by which progesterone protects cortical explants against glutamate toxicity.

Methods: We used organotypic explants to investigate the mechanism by which progesterone protects cortical explants against glutamate toxicity. Western blot analysis was performed to determine PARP cleavage, phospho-AKT, phosphoERK, ERK and Akt protein levels. Assessment of cell death was accomplished using the LDH membrane integrity assay and complemented with evaluation of PARP cleavage. An enzyme linked immunosorbant assay was used to detect and quantify cellular BDNF levels.

Results: We evaluated the ability of progesterone to protect cortical explants against glutamate toxicity using a LDH release assay complemented with the assessment of PARP cleavage (an index of caspase-mediated cell death), using Western blot analysis. A dose response analysis revealed that 10 mM glutamate for 6hr was effective at eliciting neurotoxicity. Progesterone (100 nM) pretreatment for 24 hours in turn, protected against the toxic consequences of glutamate on LDH release. In non-glutamate treated cultures, progesterone was found to elicit a two-fold increase in BDNF levels relative to untreated controls, suggesting that progesterone□s protective effects could be mediated by the pro-survival growth factor, BDNF.

Conclusions: Progesterone elicits the phosphorylation of ERK and Akt, two key effectors of signaling pathways relevant to the promotion of cell survival. Pretreatment with progesterone is protective against glutamate toxicity, and such protection may be afforded by the ability of progesterone to elicit an increase in cellular BDNF content.

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EFFECT OF PRESENILIN-1 ON INTRACELLULAR CALCIUM CHANNELS

Purpose: Alzheimer's disease (AD) is characterized by changes in the calcium (Ca^{2+}) homeostasis in neurons. Mutations in the presenilin genes are linked to early onset AD by sensitizing cells to apoptosis and causing changes in intracellular Ca^{2+} homeostasis. One of cytosolic Ca^{2+} sources is from intracellular Ca^{2+} stores such as the endoplasmic reticulum (ER) by activation of intracellular Ca^{2+} channels (ICCs). Previous reports have identified a protein-protein interaction of ryanodine receptors (RyRs) with mutated presenilin-1 (PS-1) resulting in an increased Ca^{2+} release in neurons (1, 2). The aim of this study is to identify the specific regions of PS-1 that interact with ICCs and the mechanism of action by which PS-1 modulates the activity of ICCs and determine possible signaling pathways involved in PS-1 regulated Ca^{2+} homeostasis during neurodegeneration in both AD and aging.

Methods: cDNA fragments of mouse PS1 were isolated from a mouse P19 embryonal pluripotential carcinoma cell line by RT-PCR. Site-directed mutagenesis of the respective PS-1 domains was carried out to determine changes in interaction between PS-1 and ICCs. Cytosolic loop and amino-terminal domains of mouse PS1 were expressed in a prokaryotic expression system as GST-fusion proteins and FPLC-purified. ER microsomes containing different ICCs were obtained from mouse tissue by differential ultracentrifugation. Co-immunoprecipitation assays were used to detect protein-protein interactions of PS-1 and ICCs. Ca^{2+} release from ER microsomes was measured by spectrofluorimetry to assess RyR channel activity in the presence or absence of PS-1.

Results: cDNA fragments of the cytosolic loop and amino-terminal domains from mouse PS1 were cloned into prokaryotic expression vectors. Cytosolic loop and amino-terminal domains of mouse PS1 with and without AD-related mutations were purified as GST-fusion proteins and FPLC-purified with and without GST-tag.
Protein-protein interaction of PS-1 fragments with ICCs and effects of these interactions on Ca^{2+} homeostasis in neurons were determined.

Conclusions: PS-1 is a potential regulator of ICC function through direct protein-protein interaction in the ER membrane.

References: 1. Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP. Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. J Biol Chem. 2000 Jun 16;275(24):18195-200.

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THE ROLE OF THE ANDROGEN RECEPTOR IN MEDIATING DIHYDROTESTOSTERONE INDUCED CELL SURVIVAL

Purpose: Dihydrotestosterone (DHT) is a major metabolite of testosterone, and is involved in various biological functions in both males and females, ranging from its role in the development of secondary sexual characteristics to neuroprotection. The latter serves as the principal focus of our investigation. Androgens have been shown to protect cells against various insults, including beta-amyloid, a protein linked to the pathobiology of Alzheimer□s disease. What is unclear, however, is whether androgens, like DHT, mediate their protective effects through the androgen receptor or some alternative mechanism, such as that which has been identified for estrogens.

Methods: Using a glial cell line (C6 glioma), a pharmacological antagonist to the AR, and the Androgen Receptor Knockout (ARKO) mice, we sought to identify the role of the androgen receptor on indices of cell viability. The Calcein-AM assay was used to evaluate C6 cell survival. Iodoacetic acid (IAA) served as the insult and cell viability was evaluated in the presence/absence of DHT and Nilutamide (AR antagonist). In addition, as a possible mediator of neuroprotection, we also evaluated the levels of both the pro- and mature forms of the neurotrophin, brain derived neurotrophic factor (BDNF), in mice that are deficient in the AR. Total BDNF levels in wildtype and ARKO mice were analyzed by the Promega Immunoassay system, and the pro- and mature BDNF levels were compared using the Western blot analysis.

Results: We found that DHT elicits a modest protection of C6 cells, but surprisingly, Nilutamide greatly enhanced the ability of DHT to protect. Compared to wild type mice, total BDNF levels (proBDNF + mature BDNF) in the cortex and hippocampus of ARKO mice were reduced by approximately 30%. Western blot analysis verified that this change was due primarily to a reduction in proBDNF.

Conclusions: Given that proBDNF may mediate cell death (via a p75 neurotrophin receptor-mediated mechanism), we believe that these data are consistent with the hypothesis that the expression of AR may mediate negative effects of androgens on the cell, or alternatively, the absence or antagonism of the AR is survival enhancing. [This work was supported in part by: The National Institutes on Aging (NIA) 1 PO1AG22550-01 (Project 4) and 1 RO3 AG023330-01 awarded to M.S.]

Sponsor: *National Institutes on Aging (NIA)*

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DEREGULATION OF PROTEIN KINASE B INFLUENCED ANTIAPOPTOTIC SIGNALING BY PROTEIN KINASE C IN BREAST CANCER CELLS

Purpose: We have previously shown that novel protein kinase C (nPKC) isozymes, such as nPKC-epsilon, negatively regulate TNF-induced apoptosis in breast cancer cells although the level on nPKCs did not correlate with cellular sensitivity to TNF. In the present study, we examined if the level/activation status of Akt/PKB influences antiapoptotic signaling by nPKC-epsilon.

Methods: PKB expression and activation level of the breast cancer cells were determined using Western blot analysis. Cell death was detected using Flow cytometric analysis after cells were treated with TNF with or without Ly294002, the inhibitor of PI3K/PKB pathway. Constitutively active and dominant negative PKB was transfected into MCF-7 cells or BT-20 cells, cell death mediated by TNF was detected using Flow cytometric Analysis.

Results: While MCF-7 cells overexpressed PKB, BT-20 and SKBR-3 cells expressed constitutively phosphorylated PKB, and MDA-MB-231 cells expressed unphosphorylated PKB. Ly294002, an inhibitor of PI-3 kinase, induced cell death in SKBR-3 cells, which contained little nPKCs. Although Ly294,002 by itself had only a modest effect on cell death in BT-20 and MCF-7 cells, it potentiated sensitivity of these cells to TNF. In contrast, Ly294002 either alone or in combination with TNF had little effect on cell death in MDA-MB-231 cells. Introduction of constitutively active Akt protected TNF-induced cell death in MCF-7 cells and inactivation of PKB by the introduction of dominantnegative PKB potentiated TNF-induced cell death in BT-20 cells.

Conclusions: These results suggest that the status of PKB in breast cancer cells influences antiapoptotic signaling by PKC.

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GENDER DIFFERENCES IN THE ONSET AND SEVERITY OF THE SYMPTOMS OF CANCER-RELATED ANEMIA

Purpose: Baseline differences in red blood cell mass between men and women may need to be considered in the management of cancer-related anemia with recombinant human erythropoetin (rHuEPO) for health-related quality of life (HRQoL) benefit.

Methods: Valid information on patient fatigue was collected from 197 patients with solid tumors via the Functional Assessment of Cancer Therapy Anemia (FACT, An) subscale questionnaire. Additional patient information including diagnosis, age, gender, Eastern Cooperative Oncology Group (ECOG) performance status, current therapy, and hemoglobin level was recorded and considered for a possible role in patient fatigue.

Results: Hemoglobin concentration was positively correlated with FACT-An subscale score ($p < .001$), as were improvements in ECOG performance status by regression analysis ($p < .001$). Patients receiving chemotherapy experienced greater fatigue than those who were not by independent t-test ($p < .01$). Regression analysis revealed that patient age did not have a statistically significant impact on FACT-An subscale score ($p = .07$). Controlling for the potentially confounding effects of ECOG performance status and current therapy, analysis of covariance found that male patients experienced a significantly greater level of fatigue than did females when considering respondents of all hemoglobin values ($p < .05$). However, this difference was only significant among patients in the 10.0 - 13.0 g/dL hemoglobin range ($p < .01$) with diminishing disparity at greater hemoglobin levels.

Conclusions: Men begin to experience the symptoms of cancer-related anemia at relatively greater hemoglobin values than do females, and at equivalent hemoglobin concentrations, men report greater fatigue. These data suggest that male patients require earlier intervention and the maintenance of hemoglobin concentration at greater target levels when using rHuEPO for HRQoL benefit.

Sponsor:

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THROMBIN UPREGULATES CYCLOOXYGENASE-2 EXPRESSION IN DU-145 PROSTATE CANCER CELLS

Purpose: This study was to determine the regulatory mechanism of cyclooxygenase-2 (COX-2) expression by thrombin stimulation in DU-145 prostate cancer cells.

Methods: Expression of thrombin receptors PAR-1, PAR-3 and PAR-4 in DU-145 prostate cancer cells was determined by RT-PCR and flow cytometry. DU-145 cells were stimulated with increasing concentration of thrombin and COX-2 expression was determined by semi-quantitative RT-PCR. Specific PAR-activating peptides were used to determine which PAR mediated COX-2 mRNA upregulation in DU-145 cells. Increase of COX-2 mRNA by 200 uM TFLLRN (AP-1), TFRGAP (AP-3), and AYPGKF (AP-4) was analyzed by semi-quantitative RT-PCR.

Results: We determined the expression of the thrombin receptors PAR-3 and PAR-4, in addition to PAR-1, in DU-145 prostate cancer cells. Treatment of DU-145 cells with thrombin caused increase in COX-2 mRNA expression which was abolished by hirudin, an inhibitor of thrombin proteolytic activity, suggesting that proteolytic activity of thrombin is important in COX-2 mRNA upregulation. Stimulation with a specific PAR-1 activating peptide significantly upregulated COX-2 expression while PAR-4 activating peptide did not.

Conclusions: Thus, this study demonstrates that DU-145 prostate cancer cells express thrombin receptors PAR-1, PAR-3, and PAR-4, and that thrombin stimulates upregulation of COX-2 expression most likely via PAR-1-mediated pathway.

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COMPARISON OF DIFFERENT LIPID FORMULATIONS OF RECONSTITUTED HDL (RHDL) DRUG DELIVERY VEHICLES FOR CANCER CHEMOTHERAPY

Purpose: Our purpose was to maximize the loading of reconstituted HDL (rHDL) with hydrophobic cancer chemotherapeutic agents, e.g., paclitaxel (PX), and to enhance their delivery to metastatic tumors and cytotoxicity of these agents. Since a number of cancer chemotherapeutic agents (CTA) are hydrophobic, the further development of rHDL to enhance solubility of CTA and also target metastatic cancer would facilitate cancer chemotherapy.

Methods: rHDL was prepared by the cholate dialysis method using apolipoprotein A-I (apoA-I), 14C-paclitaxel (14C-PX), various quantities of phosphatidyl choline (PC) and cholesterol esters (CE), and other phospholipids. Following removal of cholate by dialysis, these preparations were ultacentrifuged and the rHDL/drug was tested for cytotoxicity against tumor cell lines using the MTT assay.

Results: Varying PC concentrations(0-21mg), the incorporation of PX into rHDL increased in a linear fashion until it plateaued at 15-18 mg PC. Following ultracentrifugation, the rHDL became less dense with increasing PC while electrophoretically rHDL decreased in mobility. The optimal CE content was 0.15 mg CE while increasing the content of CE (0-2 mg) lead to a decrease in PX incorporation but little change in electrophoretic mobility. Varying the PX demonstrated optimal incorporation at 2 mg PX (ratio of PX/apoA-I of 0.4). Cytotoxicity studies with varying PC and CE formulations of rHDL showed rHDL/PX was more effective than free PX but little difference between rHDLs with varying CE and phospholipid composition was found.

Conclusions: Based on recovery of essentially all 14C-PX in the HDL following ultracentrifugation, the rHDL/drug preparation following dialysis to remove cholate can be directly used; phospholipid at 15-18 mg (ratio of PC/apoA-I of 3) gives optimal loading of PX into rHDL in the presence of cholesterol ester (CE) of 0.15 mg; maximal loading of 14C-PX occurs at 2 mg PX at (ratio of PX/apoA-I of 0.4); and cytotoxicity of rHDL/PX is a function of PX content and not PC or CE content. These findings clearly point to optimal formulations of rHDL for delivery of hydrophobic drugs to the systemic circulation and for targeting metastatic tumors.

Sponsor:

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EGCG AND IT'S ROLE IN PROSTATE CANCER ANGIOGENESIS

Purpose: Epigallocatechin gallate (EGCG), the major polyphenol in green tea, has been shown to inhibit cell proliferation, and to induce apoptosis in various tumor cells, including prostate cancer cells. In this study, we investigate the effects of EGCG on HIF-1a, a key transcription factor in hypoxia induced gene expression , which includes vascular endothelial growth factor (VEGF), the best known angiogenic factor, in prostate cancer cells (PC-3ML cells).

Methods: Hypoxic regulation of hypoxia response element (HRE)-mediated transcription and VEGF transcription in PC-3ML prostate cancer cells was tested by using transient transfection with pGL3-6x HRE and pGL3-VEGF plasmids, respectively, followed by luciferase analysis 20-24 hour post transfection. To test the regulatory effect of EGCG on HRE-mediated and VEGF transcription, PC3-ML cells were treated with increasing concentration of EGCG, genistein, and MG-132, 4 hrs after transient transfection. To determine the effect of EGCG on HIF-1a protein expression, PC-3ML cell extracts were prepared after treatment with EGCG, genistein, MG-132, and CoCl2 (chemical hypoxic inducer and positive control for HIF-1a protein induction), followed by immunoblot analysis.

Results: Hypoxic treatment induced a marked upregulation of HRE-mediated transcription by 16 fold, and VEGF transcription by 1.4 fold. To our surprise, EGCG upregulated HRE-mediated and VEGF transcription in PC-3ML cells in a dose-dependent manner, while genistein, a soy isoflavon and tyrosine kinase inhibitor, inhibited both transcriptions. MG132, a proteasome inhibitor, increased both HRE-mediated and VEGF transcription, in agreement with previous results in which it induced HIF-1a protein. Immunoblot analysis showed 3-fold increase in HIF-1a protein expression in EGCG-treated cells, compared to control PC-3ML cells.

Conclusions: In this study, we determined for the first time that EGCG, a major polyphenol in green tea, upregulated HRE-mediated and VEGF transcription in PC-3ML prostate cancer cells. Previously, EGCG has been shown to possess a proteasome inhibitory activity. Thus, we speculate that EGCG prevents degradation of HIF-1a protein through its proteasome inhibitory activity, resulting in increased transcription of hypoxia response genes such as VEGF via HRE-mediated pathway.

Sponsor: *NIH grant # 1R21CA102382-01 and Tobacco Research Grant from UNTHSC to Myoung H. Kim*

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HORSE SERUM HIGH DENSITY LIPOPROTEIN (HDL) AS A DRUG DELIVERY VEHICLE

Purpose: The purpose of this research is to evaluate horse serum HDL as a drug transporter, including the stability of the drug/HDL preparations. Our laboratory has developed a novel drug delivery system based on reconstituted and native HDL complexes that is highly effective in enhancing the solubility of hydrophobic drugs and may have particular utility in cancer chemotherapy. Horse serum because of its wide availability, high HDL content and the absence of cholesteryl ester transfer protein (CETP) may serve as an attractive model system for studying HDL/drug complexes and their uptake by tumor cells, and to study the transfer of hydrophobic drugs to other lipoprroteins. There are two mechanisms of cholesteryl ester transfer that can be studied independently using native horse plasma and horse plasma supplemented with partially purified CETP from human plasma.

Methods: Horse serum HDL was isolated by dodecylamine (DDA)-agarose chromatography followed by anti-serum albumin immunoaffinity chromatography. The DDA affinity chromatography approach was considerably more efficient than the conventional ultracentrifugation procedure. The HDL (isolated by the DDA procedure was characterized regarding its chemical composition and molecular size. Horse HDL/drug complexes were prepared by incubating the isolated horse serum HDL with dilauroyl fluoresceine (DLF), a hydrophobic compound used to study the movement from HDL to other lipoproteins and uptake studies with cancer cells. DLF is a good surrogate for many anti-cancer drugs because of its low water solubility and was used to study stability of the HDL/drug complex and its uptake by cancer cells.

Results: The DLF rom drug/HDL complexes was efficiently taken up by an ovarian cell line (HGL5), breast cancer (MCF7), prostate cancer (DU145) and ovarian cancer (OV1063) cells.

Conclusions: These data show that: 1) the HDL delivery vehicle is likely to be effective as a drug delivery agent against cancer cells and 2) that dilauroyl fluoresceine is a suitable model compound for the study of the interactions between drugs and cancer cells and 3) There is movement of CE form HDL to LDL but not DLF when the HDL/DLF complex is incubated with human serum.

Sponsor:

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HIGH DENSITY LIPOPROTEIN COMPLEXES AS DELIVERY VEHICLES FOR PACLITAXEL CANCER CHEMOTHERAPY.

Purpose: A new approach for drug delivery to malignant tumors has been developed by encapsulating chemotherapeutic agents into reconstituted high density lipoprotein (rHDL). Although chemotherapy is widely used in treating cancer, drug resistance developed by some tumors, poor water solubility of drugs, and toxic side effects present serious obstacles during the treatment of cancer patients. The drug delivery system utilizing rHDL particles has marked advantages over an alternative, liposomes, because the rHDL particles are smaller in size and their core components are rapidly internalized by receptors in tumor tissue. The chemotherapeutic agent chosen for our studies is paclitaxel (Ptx) as it is frequently used in the treatment of cancer. However, the therapeutic impact of Ptx is less than one would expect due to its poor water solubility and its potential for inducing resistance in tumors during therapy. TAXOL®, the most commonly used formulation for the i.v. administration of Ptx, contains Cremophor EL, an emulsifier associated with a number of toxic side effects.

Methods: Recently we have developed new formulations of rHDL/paclitaxel (rHDL/Ptx) complexes with no cremophor or substantially less cremophor than in our earlier studies. rHDL/Ptx was prepared by three different procedures: cholate dialysis, sonication, and sonication with Vitamin E /TPGS as an emulsifier. The anticancer activity of these rHDL/Ptx complexes were measured by the cell viability studies using MTT assay and calcein AM assay.

Results: The new rHDL/Ptx formulation has twice the cytotoxicity against cancer cells compared to free TAXOL®. In vivo studies showed that the residence time of Ptx in circulation of mice was extended by incorporating the drug into rHDL. All these 3 formulations are quite capable of encapsulating paclitaxel,and showed enhanced cytotoxicity towards the cancer cell lines compared to paclitaxel.

Conclusions: rHDL formulation will enhance the efficacy of currently employed chemotherapeutic agents by diminishing their propensity for inducing drug resistance and their systemic toxicity. Other potential advantages of the new drug delivery system involve targeting via chemical modifications of the protein or lipid constituents of the HDL complex, in addition to overcoming the resistance of certain tumors against anti-cancer drugs.

Sponsor: *DOD/ Idea development*

207

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PILOT STUDY TO DETERMINE THE EFFECTS OF DRONABINOL ON HOT FLASH RELIEF IN BREAST CANCER PATIENTS

Purpose: Hot flashes are a menacing problem, affecting 50-85% of menopausal women, with the incidence in breast cancer patients greater, due to antiestrogen therapy. Currently, the most effective treatment, hormone replacement therapy (HRT), is not recommended in breast cancer patients and alternative therapies are sought. These include certain antidepressants and natural supplements which result in a 55% and 30% reduction from baseline, respectively. This study was conducted to determine the efficacy of dronabinol (Marinol®) on hot flash relief. Marinol® has never been evaluated in this clinical setting.

Methods: Women diagnosed with breast cancer and suffering from hot flashes were screened for enrollment and considered eligible if inclusion/exclusion criteria were met. The European Organization for Research and Treatment of Cancer Quality of Life (QoL) (EORTC QLQ-C30) questionnaire was completed, along with a daily hot flash log for five weeks. A baseline week was recorded prior to a four week regimen of 5.0 mg of Marinol® a day, orally. Hot flash scores were calculated based on frequency and severity as previously published by Loprinzi et al (J Clin. Oncol. 2001;19:4280-4290). All adverse events and toxicities were monitored.

Results: A total of 21 patients were enrolled in this pilot study, with data available for seventeen (n=17). Of the thirteen patients that completed the study, the average hot flash scores at baseline ranged from 4.6 to 76.9, with a mean baseline change from 28.7 ± 20.5 (mean and sd) to 14.9 ± 15.3 after four weeks of treatment. The average hot flash score and daily hot flash frequency reductions from baseline were 54.8% and 43.1%, respectively. A significant statistical difference in overall QoL scores from Marinol® administration was not observed. Common side effects included headache (n=3) and insomnia (n=3).

Conclusions: Marinol® administration resulted in a significant decrease in hot flash scores, due to a decrease in frequency and severity. The percentage of reduction is comparable to standard treatments and provides a viable alternative therapy.

Sponsor: *Cancer Education and Research Foundation of Texas, Fort Worth, TX and Texas Cancer Care, Fort Worth, TX*

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A NOVEL DRUG DELIVERY SYSTEM TO REDUCE DRUG RESISTANCE IN CANCER CHEMOTHERAPY.

Purpose: Multidrug resistance (resistance to cytotoxic drugs) is a major factor in the failure of cancer chemotherapy. To reduce this problem, reconstituted high density lipoprotein (rHDL) particles with the chemotherapeutic drug encapsulated in their core have been developed, which are expected to deliver the drug to cancer cells through receptor mediated mechanisms and thus facilitate cytotoxicity. Octadecyl Rhodamine B Chloride (ORB) was used as the study compound in these studies since it is a fluorescent, poorly water-soluble compound and thus a good candidate for encapsulation into the hydrophobic core of rHDL particles.

Methods: rHDL/ORB complexes have been prepared by the sonication procedure. The evaluation of rHDL/ORB delivery system was done on drug-sensitive (OV1063) and drug-resistant (OVCAR-3 and SK-OV-3) ovarian cancer cell lines. The effectiveness of the rHDL/ORB delivery system to overcome drug resistance was done assessing the uptake and efflux studies with rHDL/ORB complex and ORB alone. We compared the amount of ORB being expelled from the drug-sensitive and drug-resistant ovarian cancer cells.

Results: The efflux of ORB from drug-resistant cells with rHDL/ORB is almost three times less than ORB alone. The selective uptake of the rHDL/ORB core components by tumor cells is an advantage in this system because the drug packed into the core of the rHDL will be taken up through receptor-mediated mechanism, thus by-passing the transmembrane efflux pumps that are responsible for multi drug resistance.

Conclusions: The uptake of ORB was more and the efflux was less when ORB was delivered to the cells as rHDL/ORB complex compared to ORB alone. Since the efflux of ORB was less using rHDL/ORB complex, the intracellular availability of ORB remains higher and hence, this may enhance the efficacy of the chemotherapeutic treatment.

Sponsor: *DOD/Idea Development*

209

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THE MOLECULAR CHARACTERIZATION OF NATURAL CYTOTOXICITY RECEPTOR LIGANDS FOR NKP46 AND NKP44

Purpose: A part of the innate immune response, bone marrow-derived natural killer (NK) cells provide a frontline defense against viral infection and tumor transformation. NK cell activation and subsequent target cell lysis is dictated by a delicate balance between inhibitory and activating signals mediated through receptor-ligand interactions. Activating signals are propagated largely through three NK cell specific natural cytotoxicity receptors (NCRs), NKp46, NKp44, and NKp30. These immunoglobulin superfamily receptors cooperate in tumor cell lysis, lack tyrosine motifs in their cytoplasmic tails, and associate with transmembrane adaptor proteins for functional activity. The NCR ligands remain unknown. Their identification and characterization will enable us to further understand the mechanisms by which NK cells mediate killing, and lead to the development of novel pharmaceutical interventions for the treatment of cancer.

Methods: NKp46 and NKp44 cDNAs were PCR amplified by using sequence specific primers and subcloned into the pGEM-T Easy Vector. Regions corresponding to the C2 domains of NKp46 and the V domain of NKp44 were subcloned into the mammalian expression vector pCD5lneg1, which contains the CH2 and CH3 regions of human IgG1. Soluble fusion proteins will be isolated and used for identification of ligands.

Results: We have cloned the cDNA for human NKp46 by RT-PCR of total RNA isolated from NK92 cells. Also, the cDNA for NKp44 was cloned from a human NK cell cDNA library.

Conclusions: We have completed the first step (subcloning of NKp46 and NKp44) required for the identification of these natural cytotoxicity receptor ligands.

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REGULATION OF DNA POL ETA AFTER UV DAMAGE IN SACCHAROMYCES CEREVISIAE

Purpose: RAD30 gene product DNA POL eta is regulated at mRNA as well as protein level

Methods: RAD30 gene was myc-tagged at carboxy terminal using a plasmid module containing 13 epitope of myc and selectable marker sequence.This helped in detecting protein in the absence of commercially available antibody . Northern blotting to see transcript levels after UV exposure. Western blotting to see protein expression after UV. Experiments including cycloheximide, a protein synthesis inhibitor, to study degradation of protein.

Results: In log phase cells message goes up with peak at 1h after UV-C treatment for 90 seconds.mRNA level falls down and maintained for 3 hours. The corresponding increase in protein expression is not seen. Protein is expressed at constitutive levels even after UV treatment.Treating log phase cells with Cycloheximide doesnot show any significant change in levels of protein. On the other hand in stationary cells protein level drops gradually from 0h to 5h after UV treatment. Further treatment with cycloheximide confirms enhanced degradation of DNA POL eta after UV insult.

Conclusions: RAD30 gene was successfully tagged at carboxy terminus enabling detection of protein.
DNA POL eta is expressed significantly without UV treatment.
The protein is quite stable in log phase cells.
Stability of the protein changec in stationary phase cells or in other words half-life of DNA POL eta is reduced indicating enhanced protein degradation.

Sponsor:

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EVALUATING THE DISPARITY OF FEMALE BREAST CANCER MORTALITY AMONG RACIAL AND AGE-SPECIFIC GROUPS: A SPATIOTEMPORAL ANALYSIS

Purpose: The literature suggests that the distribution of female breast cancer mortality demonstrates spatial concentration by race and ethnicity. There remains a lack of studies on how age-specific mortality burden may impact racial groups across space and over time. Particularly of interest is the age group (50-69), which is recommended for mammography screening, while age group (40-49) is controversial. The present study evaluated the geographic variations in breast cancer mortality in Texas females in two age groups with reference to three predominant racial groups (non-Hispanic White, Black, and Hispanic females) over a twelve-year period. The goal of this study is to clarify whether the spatiotemporal trend might place an uneven burden on particular racial groups in any specific age groups, and also whether the excess trend has persisted into the current decade.

Methods: The Spatial Scan Statistic was employed to examine the geographic excess of breast cancer mortality by race in Texas counties between 1990 and 2001. The statistic was conducted with a scan window of a maximum of 90% of the study period and a spatial cluster size of 50% of the population at risk. The next scan was conducted with a purely spatial option to verify whether the excess mortality persisted further.

Results: The spatial scan identified three regions in the Northwest, six regions in the Gulf Coast and in South Texas with breast cancer mortality excess in both non-Hispanic Whites and Hispanic populations. In the 40-49 age group, the most likely excess mortality occurred with a relative risk of 1.83 (p=0.004) between 1990 and 1994 for Hispanics. For the age group 50-69, a relative risk of 1.21 (p=0.001) occurred for Non-Hispanic White between 1990 and 1995 and for Hispanics a relative risk of 1.38 (p=0.01) took place between 1993 and 2000 in the Southeast and Gulf Coast Texas and this was the most probable region of excess mortality with a statistical significance.

Conclusions: Spatiotemporal variations in breast cancer mortality affected racial and age groups at varying levels. There is supporting evidence of a persistent spatiotemporal trend of excess mortality into the present decade for Hispanics. As regards the two age groups examined in this study, Non-Hispanic Whites and Hispanics aged 40-49 in the Northwest Texas and Hispanics aged 50-69 in the East and Gulf Coast Texas, carried the highest burden of mortality, as evinced by spatial concentration and temporal persistence.

Sponsor:

300

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DYNAMIC CEREBRAL AUTOREGULATION DURING EXERCISE IN HUMANS

Purpose: The purpose of this study is to evaluate the influence of exercise on dynamic cerebral autoregulation (CA) and its interaction with changes in dynamic arterial baroreflex control of arterial blood pressure during exercise.

Methods: Each subject performed three 20 min bouts of exercise at a steady-state heart rate (HR) of 90, 120, and 150 beats/min. Transfer function gain, phase shift and coherence between changes in mean arterial pressure (MAP) and middle cerebral artery mean blood flow velocity (MCA Vmean) assessed the frequency components of the dynamic CA. To estimate dynamic arterial baroreflex function, transfer function gain between changes in MAP and HR or total vascular resistance (TVR) was calculated.

Results: Transfer function gain and phase shift between MAP and MCA Vmean, for each frequency range analyzed, remained stable and did not differ significantly from the resting values and indicate a preservation of dynamic CA during exercise. Only the coherence between MAP and MCA Vmean decreased during heavy exercise ($P<0.05$). In contrast, transfer function gain between MAP and HR or TVR decreased ($P<0.05$) and was associated with the decrease in the coherence between MAP and MCA Vmean with increasing workload.

Conclusions: These findings suggest that as the workload increased, dynamic regulation of cerebral blood flow used more of the functional CA reserve because baroreflex control of arterial blood pressure regulation was attenuated.

Sponsor:

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FOCALSEAL-L ABSORBABLE SEALANT REDUCES AIR LEAKS INCURRED DURING PULMONARY RESECTIONS.

Purpose: Approximately 260,000 lung surgery procedures, primarily for the treatment of lung cancer resulting from years of smoking, are performed worldwide each year. A common and potentially dangerous outcome in pulmonary surgery, air leaks can introduce a number of problems, including prolonged chest drainage, time-to-ambulation, increased morbidity due to prolonged air leaks, and extended postoperative stay.
FocalSeal-L Sealant is intended for use as an adjunct to standard closure of visceral pleural air leaks incurred during elective pulmonary resection by placing a thin, adherent coating of biocompatible and absorbable hydrogel onto tissue.

Methods: Twenty patients undergoing thoracotomy were randomized intraoperatively in a 1:1 ratio to receive surgical sealant applied to sites at risk for air leak after standard methods of lung closure (treatment group) or to have standard lung closure only (control group). The study and control groups were well matched demographically. The primary outcome variable was incidence and duration of post operative air leaks. Secondary outcome variable was duration of chest tube drainage.

Results: A significantly higher percentage of treated patients than the control patients remained free of air leaks throughout hospitalization; 80% vs. 20%, $p=0.01$. Air leak duration was greatly reduced from 2.6 (mean) to 0.3 (mean) days in those patients that incurred a post operative leak. Additionally chest tube drainage was required only 1.8 days (mean) vs. 4.7 days (mean), $p=0.01$. In the treatment group, trends were observed for reduced time to chest tube removal and earlier discharge.

Conclusions: Per Diem costs were calculated from recent data of 1,577 Medicare discharges resulting in a mean cost per day of $1,119 for DRG 75. Incorporation of FocalSeal-L tissue sealant can result in approximately $3,357 savings per hospital stay.

This novel adjuvant treatment for pulmonary surgery can result in decreased patient morbidity and significant reduction in hospital stay length and associated costs.

Sponsor:

302

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CEREBRAL AUTOREGULATION (CA) DURING SUSTAINED AND TRANSIENT SYSTEMIC HYPOTENSION

Purpose: Cerebral autoregulation (CA) is mediated by myogenic and metabolic mechanisms. The purpose of this study was to test the hypothesis that the mechanism of CA could be different in response to the stimulus elicited by sustained and transient hypotension.

Methods: Eight healthy men (26+/-2 yr, 76+/-3 Kg, 175+/-3 cm) gave written consent to participate in the study that was approved by IRB at UNTHSC. During the experiment heart rate (HR, electrocardiograph), stroke volume (SV, impedance), arterial blood pressure (ABP, tonometey), mean middle cerebral arterial blood flow velocity (VMCA, transcranial Doppler), systemic arterial O2 saturation (SaO2, Oximeter), and regional cerebral O2 saturation (RcO2, near-infrared spectroscopy) were continuously monitored. Sustained and transient systemic hypotension was elicited by lower body negative pressure (LBNP) and bilateral thigh cuff deflation following supra-systolic occlusion, respectively.

Results: LBNP 50 torr significantly decreased VMCA by -10+/-2%, from 64+/-3 to 58+/-4 cm/s, with maintained mean ABP (baseline 81+/-2 mmHg). This VMCA change was associated with a significant decrease (-3.3+/-0.6%) in RcO2 (from baseline 71+/-2%) without change in SaO2. Time for maximal decrease (Dmax) in mean ABP (-16+/-1 mmHg) and VMCA (-12+/-1 cm/s) elicited by transient systemic hypotension at rest were1.8+/-02s and1.5+/-0.2s, respectively. The time for Dmax in RcO2 (-3.5+/-0.4%) was (3.8+/-0.6s) significantly longer, which was preceded by recovery process of VMCA.

Conclusions: Our date indicated that metabolic mechanism was predominant for CA during LBNP elicited sustained systemic hypotension, whereas myogenic mechanism initiated CA during transient systemic hypotension.

Sponsor: *NIH Grant HL65613*

303

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HYPORESPONSE TO STATINS AND LDL-C FLUCTUATION IN TEXCAPS COHORT.

Purpose: Statins are the most widely used lipid-lowering agents to lower LDL-C. In the Texas coronary atherosclerosis prevention study (TEXCAPS), the mean LDL-C reduction with lovastatin was 25% from baseline to a LDL-C of 115 mg/dl. Our objective was to characterize hyporesponders (n=152) to lovastatin in this cohort based on their decreased response to lovastatin after 18 weeks of initial therapy (< 15% LDL reduction).

Methods: We recruited participants (n = 25) and monitored the response in a crossover design consisting of three therapy periods: simvastatin, simvastatin-phytosterol, and double the dose of simvastatin.
In searching for an explanation of the clinical trial results, we retrospectively reviewed the TEXCAPS clinical trial data for the hyporesponders (n=152) for response at end of trial with the expectation of finding most had responded to long term therapy.

Results: After 6 weeks on simvastatin (10 mg), the group's LDL-C had decreased >15% with no further change noted with addition of phytosterol or doubling the dose of simvastatin. The >15% response in LDL-C lowering to this statin was unexpected as well as the lack of response to the additional dose of statin/phytosterol.
From the retrospective chart review, we found that 60.5% (n=92) failed to respond to long term therapy with lovastatin (40 mg). We also noted that LDL-C fluctuated in this cohort. Over the study period (4.0±1.4 years) of these enrollees, LDL fluctuated >15% (n=3.8 fluctuations, 32.3% of total visits) between visits (mean visits =11.6).

Conclusions: 1) Identification of hyporesponders to lovastatin was not confirmed by using a different statin, simvastatin. 2) Subjects responded to simvastatin as responders but doubling dose of simvastatin or phytosterol had no additional lowering effect. 3) Over the study period, LDL fluctuates >15% in this selected cohort on approximately 1/3 of the visits. Whether this cycle of LDL fluctuations is associated with increased incidence of CAD remains to be established.

Sponsor:

304

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EVALUATION OF AIR FORCE/TEXAS CORONARY ATHEROSCLEROSIS PREVENTION STUDY (AFCAPS/TEXCAPS) COHORT USING NATIONAL CHOLESTEROL EDUCATION PROGRAM-ADULT TREATMENT PANEL III GUIDELINES.

Purpose: The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) reported diet with lovastatin , 20-40 mg, reduced the risk for the first coronary event by 37%. Our objective was to reevaluate the AFCAPS/TexCAPS cohort utilizing the new National Cholesterol Education Program-Adult Treatment Panel III guidelines (NCEP-ATP III) since the original study was based on ATP II guidelines.

Methods: A retrospective chart review of the AFCAPS/TexCAPS cohort was done. The main outcome measures were event rates of first acute major coronary events (ACME) stratified by NCEP-ATP III guidelines and target goals.

Results: There was a 39% (21%,53%; 95% CI; p<0.001) reduction in ACME of treated subjects in the 65% of the cohort eligible for drug therapy and a 34% (-9%,60%; p=0.108) reduction in the 35% who would not be eligible for drug therapy. The Framingham risk score overestimated the incidence of ACME in the placebo group by 22% for males and 49% for females across all risk categories; if excluded from endpoint, overestimate of unstable angina became 43% & 94%, respectively. Evaluation of other components of the NCEP-ATP III guidelines included: 44% (27%,58%; p<0.001) reduction in ACME in subjects with baseline HDL-C < 40 mg/dl; 40% (17%,56%; p=0.002) reduction in subjects with metabolic syndrome; 15% (-134%,69%; p=0.748) reduction in diabetics; and 38% (12%,56%; p=0.007) reduction in subjects with neither metabolic syndrome nor diabetes. ACMEs (21%) occurred in subjects who would not be eligible for drug therapy and in 48% of those subjects with metabolic syndrome. A prespecified Cox backward stepwise regression model identified outcome predictors and logistic regression models examined the relation between lipid variables and ACMEs. Baseline ApoB appeared to be a better predictor of ACMEs than non-HDL-C. Treatment to a target LDL-C goal < 130 mg/dl suggested a non-significant trend to greater benefit.

Conclusions: The NCEP-ATP III guidelines do a better job than ATP II at defining the at-risk population in the AFCAPS/TexCAPS cohort. Still, 21% of the ACMEs were missed with these new guidelines. The presence of metabolic syndrome may help define additional risk in individuals who would not be eligible for treatment with a medication under ATP III guidelines. Further evaluation of the accuracy of the predictive value of the Framingham risk score and the LDL-C target goal of < 130 mg/dl for those at intermediate risk need to be reconsidered pending results from future trials.

Sponsor:

305

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PULSE TRANSIENT TIME (PTT) DURING LOWER BODY NEGATIVE PRESSURE (LBNP)

Purpose: The purpose of this study was to compare the change in PTT to middle cerebral artery (MCA) and to radial artery (RA) during LBNP.

Methods: Eight healthy men (26±2 yr, 76±3 Kg, 175±3 cm) gave written consent to participate in the study that was approved by IRB at UNTHSC. PTTMCA and PTTRA were calculated from the distance between R wave and peak pulse wave of MCA blood flow velocity (VMCA, transcranial Doppler) and peak pulse wave of RA blood pressure (tonometry), respectively.

Results: PTTMCA was consistently shorter than PTTRA at rest (201+/-10 vs 289+/-9 ms) and during LBNP (212+/-10 vs 292+/-10 ms). However, LBNP -50 torr prolonged PTTMCA by 6.1+/-2.1% (P=0.02) without altering PTTRA. Heart rate (+15.6% from 63+/-3 bpm), RA pulse pressure (delta P, -24+/-7% from 54+/-3 mmHg), cardiac output (-31+/-3% from 5.03+/-0.46 L/min, impedance cardiograph), and VMCA (-10+/-2% from 64+/-3 cm/s) were significantly altered during LBNP. With significant delta P, a maintained PTTRA during LBNP indicated a decrease in systemic arterial volume (delta VA) or compliance (CA); whereas a prolonged PTTMCA implied an enhanced CA or a proportionally less delta VA in cerebral circulation.

Conclusions: We concluded that the difference between PTTRA and PTTMCA changes was attributable to a functional cerebral autoregulation during LBNP.

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MECHANISM OF CAM KINASE IIDC SILENCING OF MEF2-DEPENDENT GENE TRANSCRIPTION

Purpose: The myocyte enhancer factor-2 (MEF2) family of transcription factors regulates transcription of muscle-dependent genes in skeletal, smooth and cardiac muscle types. MEF2-mediated gene transcription is activated by calcium/calmodulin (CaM)-dependent protein kinases I and IV. Conversely, CaM KIIdC silences MEF2 transcriptional activity. In the silenced state, MEF2 is held inactive in a complex with class II histone deacetylases (HDAC) in the nucleus. When this complex is phosphorylated, HDAC is exported out of the nucleus via a 14-3-3-chaperone mechanism resulting in MEF2 mediated transcription. 14-3-3 proteins exist as dimers and all isoforms have a highly conserved region of dimerization that contains two CaM kinase II phosphorylation sites (ser60 and ser65 of b isoforms). We hypothesize that CaM kinase IIdC phosphorylation of 14-3-3b will disrupt dimer formation resulting in the return of HDAC to the nucleus and their re-association with MEF2.

Methods: We used conservative amino acid point mutation to mutate serines 60 and 65 of 14-3-3b to aspartic acid, in order to mimic a phosphorylated status of 14-3-3. Cardiomyocytes were transfected and a luciferase-based enhancer-reporter was used to define the effects that the mutated 14-3-3b proteins had on MEF2-mediated transcription. We used a kinase assay to examine the phosphorylation status of 14-3-3b in the presence of active CaM kinase II. We then went on to examine the protein interaction of mutant 14-3-3b and wild type 14-3-3b.

Results: Serines 60 and 65 of 14-3-3b were mutated to aspartates to mimic the phosphorylated state. In MEF2 enhancer-reporter assays in cardiomyocytes, overexpression of 14-3-3b mutants was able to silence MEF2-enhancer activity driven by CaM kinases I, IV and phenylephrine. Using an HDAC4 & 5-Green Fluorescent Protein fusion hybrid the cellular localization of transfected HDAC4 & 5 was followed in cardiomyocytes. The 14-3-3b mutant prevented cytoplasmic localization of HDAC4 & 5 in the presence of active CaM kinase I or IV. A mammalian two-hybrid assay revealed the 14-3-3b mutant monomers were unable to bind to wild-type monomers or other mutant monomers.

Conclusions: These data give strong evidence that the mechanism through which MEF2-dependent genes are silenced is by CaM kinase IIdC phosphorylation of 14-3-3b, allowing HDAC's to return to the nucleus to complex and silence MEF2-dependent gene transcription in cardiomyocytes.

Sponsor:

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HEMODYNAMIC RESPONSES TO INTERMITTENT HYPOXIA EXPOSURE IN TRAINED ATHLETES

Purpose: The purpose of this study was to determine whether IH causes such detrimental hemodynamic effects in trained athletes.

Methods: Twenty-two elite athletes, 11 swimmers (5m, 6f), and 11 runners (6m, 5f) were randomly assigned to either hypobaric hypoxia (HYPO: simulated altitude of 4000-5500m) or normoxia (NORM: 0-5000m) in a double blind design. Both groups rested in a hypobaric chamber 3h/d, 5d/wk for 4 wks. Hemodynamic measurements including blood pressure (BP, automated cuff), heart rate (HR, ECG), cardiac output (Qc C2H2 rebreathing), total peripheral resistance (TPR=BP/Qc), and stroke volume (SV=Qc/HR) were conducted twice before and three days after the last chamber exposure in the sitting position.

Results: We observed no changes in any variable after this exposure (Table 1, values are MEAN+/- SD).

	Normoxia n=12		Hypoxia n=10	
	Pre-Exposure	Post-Exposure	Pre-Exposure	Post-Exposure
MAP (mmHg)	90±8	86±5	87±6	85±6
TPR (dyne□s□cm-5)	1012±304		916±195	988±221	1014±309
SV (ml)	115±30	125±32	109±25	106±36
HR (bpm)	69±17	69±18	69±11	72±15
SBP (mmHg)	122±11	120±11	124±10	121±8
DBP (mmHg)	73±9	69±6	70±5	69±6

Conclusions: Intermittent hypobaric hypoxia did not alter BP or any aspect of hemodynamics in young endurance trained athletes.

Sponsor:

308

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CAROTID BAROREFLEX CONTROL OF LEG VASCULATURE AT REST AND DURING EXERCISE AFTER ORAL GLYBURIDE ADMINISTRATION

Purpose: The purpose of the current investigation was to evaluate the role of the ATP-sensitive potassium channel (KATP) in modulating carotid baroreflex-induced vasoconstriction of the leg vasculature during dynamic leg exercise.

Methods: Four subjects (24-27yrs) were instrumented with electrocardiogram, finger cuff photoplethysmography, or femoral artery catheter for measures of arterial blood pressure, and a Doppler ultrasound probe for measures of femoral artery blood velocity and diameter to determine leg blood flow and leg vascular conductance (LVC). Using the well-established neck chamber technique, neck pressures of +40mmHg were utilized to disengage the carotid baroreflex at rest and during steady-state one-legged knee extension exercise. Furthermore, subjects were administered glyburide (5mg oral) and again, underwent similar trials of neck pressure during exercise (2-4hr post-admin).

Results: At rest, neck pressure resulted in a decrease in the percentage change of LVC (-43.5+/-4%). During one-legged knee extension exercise, the percentage change of LVC was markedly reduced in the exercising leg compared to rest (-14.0+/-6%, $p<0.05$). After administration of glyburide, the percentage change of LVC was augmented (-23.3+/-7%) compared to control exercise ($p<0.05$).

Conclusions: Oral administration of glyburide appears to alter carotid baroreflex control of leg vasculature during steady-state, dynamic leg exercise. This finding is likely the result of inhibition of KATP channel activity on the smooth muscle cells of the peripheral vasculature and therefore, support work previously performed in the rat which demonstrated an important role of the KATP in modulating sympathetically-mediated vasoconstriction.

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MUTATIONS IN THE REGULATORY LIGHT CHAIN ALTER THE MYOSIN CROSS-BRIDGE CYCLING IN THE FAMILIAL HYPERTROPHIC CARDIOMYOPATHY MUSCLE

Purpose: Familial hypertrophic cardiomyopathy (FHC) is an autosomal dominant disease characterized by left ventricular and/or septal hypertrophy, myofibrillar disarray and sudden cardiac death. Mutations have been found in some of the sarcomeric proteins, one of which is the ventricular isoform of myosin regulatory light chain (RLC).
It has been shown that two mutations of this protein E22K and N47K were located close to the Ca2+ binding region of RLC. The objective of this study is to understand the role of FHC-RLC mutations in altering cardiac muscle contraction.

Methods: To achieve this goal, we measured rotational motion of a "lever arm" domain of myosin - a part that is known to rotate upon hydrolysis of ATP to cause muscle contraction. Engineered ELC were labeled with red fluorescent probeand exchanged with native light chains of cardiac myosin of heart muscle of transgenic mice carrying E22K mutation of RLC. Thus the ever arm of myosin contained RLC with hypertrophy causing mutation and fluorescent ELC. A modified confocal microscope was used to observe small populatipon of myosin molecules(~400)in situ. The cross-bridges were activated by a precise delivery of ATP from a caged precursor. The specificity of labeling was assessed by inspecting the striation pattern of muscle fibers in a confocal microscope. The anisotropy of fluorescence of cross bridges containing fluorescent light chains was measured

Results: Our preliminary results indicate that while in control fibers the rotation of the ELC is observed, no such response is observed with transgenic mutated fibers

Conclusions: These results suggest that RLC mutation alter myosin cross-bridge cycling leading to abnormal cardiac muscle contraction.

Sponsor:

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INTERMITTENT HYPOXIC TRAINING PROTECTS CANINE MYOCARDIUM FROM INFARCTION

Purpose: This investigation examined cardiac protective effects of intermittent hypoxia training (IHT).

Methods: 6 dogs underwent IHT for 20 consecutive days in a normobaric chamber ventilated intermittently with N2 to reduce FIO2 to 9.5-10 %. After 20 days of training, the resistance of ventricular myocardium to infarction was assessed in an acute experiment. The left anterior descending coronary artery (LAD) was occluded for 60 min and then reperfused for 5 h. At 30 min of LAD occlusion, radioactive microspheres were injected through a left atrial catheter to assess coronary collateral flow into the ischemic region. After 5 h reperfusion, the heart was dyed to delineate the area at risk (AAR) and stained with tetrazolium chloride to identify infarcted myocardium.

Results: During LAD occlusion and reperfusion, systemic hemodynamics and global left ventricular function were stable. Collateral flow in the inner 2/3 of the center of the AAR was averaged 0.20 ± 0.09 ml/min/g, and was less than 0.12 ml/min/g in four dogs. Infarction was not detected in four hearts, and was 1.6% of AAR in two hearts. In contrast, 4 sham trained dogs and 5 untrained dogs subjected to similar periods of LAD occlusion and reperfusion had infarction equal to 44.7 ± 4.5% and 35.2 ± 9.5% of the AAR, respectively.

Conclusions: IHT protects canine myocardium from infarction. (supported: NIH grant HL64785)

Sponsor:

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ENDOTHELIUM DEPENDENT VASCULAR RESPONSES IN DIET-INDUCED OBESE RABBITS

Purpose: The aim of the study was to investigate the role of nitric oxide (NO) on vascular reactivity in diet-induced obese rabbits using both conducting (thoracic aorta, femoral artery) and distributing (renal artery)arteries.

Methods: Isometric tension of isolated rings was recorded after preconstriction with phenylephrine, followed by relaxation to acetylcholine (ACh, 10-10-10-4 M), ACh plus the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME, 10-5 M), and sodium nitroprusside (SNP, 10-10-10-4 M).

Results: Obese rabbits were higher blood pressure (+10%) and resting tachycardia(+22%) compared to lean. Responses to ACh and SNP were not significantly different between groups. Responses to ACh+L-NAME did not differ in conducting arteries. In renal arteries L-NAME caused greater inhibition of ACh-induced relaxation in lean compared with obese (pd0.05; relaxation at 10-4 M was -17.2±13.4% and 39.4±8.0%, respectively).

Conclusions: Our results indicates that that relaxation of renal arteries in obese rabbits is less sensitive to NO inhibition, and suggests that NO-independent mechanisms may be more important for ACh-induced relaxation of renal arteries in obese rabbits.

Sponsor: *NIH HL64913*

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LOSS OF LAG RESPONSE CURVILINEARITY OF BEAT-TO-BEAT HEART RATE IN CONGESTIVE HEART FAILURE

Purpose: Heart rate variability (HRV), an index of autonomic neural control of the heart, is frequently altered in patients with congestive heart failure (CHF). This study was designed to determine, via Poincaré plot analysis, whether the inter-heart beat lag to cardiac cycle length relation is also altered in CHF. Time-domain analysis of ECG signals traditionally relies heavily on linear indices of a signal which is significantly non-linear in nature. Since lagged Poincaré plots incorporate autocovariance information, these analyses provide insights into the autonomic control of heart rate that account for both the non-linear and linear elements of the signal.

Methods: We assessed the influence of lag on estimates of Poincaré plot indices for various lengths of beat sequence in 29 subjects with CHF and 54 subjects with normal sinus rhythm. ECG recordings of these subjects were obtained from the public domain repository of PhysioNet (http://www.physionet.org). The curvilinear association of Poincaré plot indices with lag was assessed using second order polynomial regression.

Results: Traditional time-domain indices of HRV (SD1 and SD2) showed no impairment in CHF patients as compared to normal subjects. But the non-linear index SD1/SD2 was statistically significantly different in the two groups ($p<2 \times 10-6$). Normal curvilinearity of association between all Poincaré plot indices (SDLD, SD1, SD2, and SD1/SD2) and lag was significantly reduced in patients with CHF ($p<0.002$) for various beat sequence lengths (ranging from 50 to 50000), except for the relation of SD2 to lag for a beat sequence length of 50000 ($p=0.078$).

Conclusions: Non-linear indices of HRV behave differently in CHF as compared to normal sinus rhythm. In this group of subjects, despite normal time-domain HRV, the Poincaré plot data indicate an impairment in the CHF patients. We hypothesize that these results point towards the existence of an autonomic "memory" in normal subjects which may be impaired in CHF.

Sponsor:

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STEROL-RICH MEMBRANE RAFTS REGULATE EXPOSURE OF PLATELET BINDING SITES FOR FACTOR IXA.

Purpose: Sterol-rich membrane microdomains (lipid rafts) have been reported to regulate cell activation. Platelet membranes contain such microdomains enriched upon activation in the glycoprotein GPIb complex as well as in signaling molecules. Platelet activation leads to exposure of a procoagulant surface for assembly of the intrinsic factor X (FX) activation complex. This study was designed to examine if disruption of lipid rafts affected platelet surface assembly of the FX-activating complex.

Methods: First, kinetic assays were used to investigate the functional activity of MbCD-treated platelets. Gel-filtered platelets were treated with 10 mM methyl beta-cyclodextrin (MbCD) to deplete cholesterol from cell membranes before further washing and gel-filtration. Washed MbCD-treated and control platelets, both activated with the thrombin receptor peptide, were compared in kinetic studies of factor Xa generation in the presence of FIXa, FVIIIa and FX. Second, flow cytometry was used to investigate the FIXa-binding capacity of MbCD-treated platelets. SFLLRN-activated, MbCD-treated, and unactivated platelets were incubated with 10nM factor IXa and biotin-labeled anti-factor IXa/streptavidin-PEcy5 and/or FITC-anti GPIba and/or FITC-annexin V before analysis for bound fluorescence.

Results: Kinetic studies surprisingly showed that platelets treated with MbCD exhibited marked increases in functional factor-X-activation complexes. In FX titrations, Vmax values were 2-3-fold increased with MbCD-treated platelets with no alteration of Kmapp compared with control activated platelets.

Flow cytometry studies showed MbCD-treated platelets exhibited marked increases in factor-IXa binding sites and aminophospholipid exposure (annexin V binding) independently of the action of a platelet agonist (ie, SFLLRN). Consistent with our previous observations, a mean 7% of SFLLRN-activated platelets showed a high density of FIXa bound. In contrast, a mean 50% of MbCD-treated platelets showed the same high density of FIXa binding sites. Moreover, MbCD-treatment resulted in a 10-fold increase in the number of platelets binding annexin V.

Conclusions: These results suggest that disruption of lipid rafts, depletion of platelet cholesterol or some other activating effect of MbCD on platelets recruits platelets to expose aminophospholipids and factor-IXa binding sites and assemble functional factor-X activation complexes.

Sponsor: *Temple University School of Medicine*

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EFFECT OF OBESITY AND HYDRALAZINE TREATMENT ON DIURNAL RHYTHMS OF BLOOD PRESSURE AND HEART RATE

Purpose: Obesity results in both hypertension and loss of heart rate (HR) and blood pressure (BP) diurnal rhythms. Hypertension itself is associated with loss of HR and BP diurnal rhythms. Whether BP control in obesity restores diurnal rhythms is unknown. We tested whether BP control using hydralazine reversed loss of diurnal patterns during 12 wks of developing obesity in rabbits.

Methods: Female New Zealand white rabbits were divided into lean control (LC, n=8), lean hydralazine-treated (LH, n=10), obese control (OC, n=8) and obese hydralazine-treated (OH, n=11) groups. Obese rabbits ate an ad lib high fat diet; lean rabbits ate a maintenance diet. BP was monitored from 1100-0700 h daily using telemetry. Hydralazine treatment (6-14 mg/kg/d) began during wk 2 of the dietary protocol. To evaluate diurnal rhythms, night (0200-0700 h) values were subtracted from day (1100-0400 h) values; groups were compared using one-way ANOVA.

Results: Groups did not differ in day-night BP or HR during the control period. After 12 wks of high-fat feeding, day-night BP was reduced in OC and OH, suggesting that there was no nighttime dipping of BP. Day-night HR was lower in OH compared with LC and LH.

Conclusions: These data suggest that obesity□s role in the loss of HR and BP diurnal rhythms is independent of hypertension.

Sponsor:

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EFFECTS OF A NOVEL ALLELE FOR EXTRACELLULAR SUPEROXIDE DISMUTASE ON THE TISSUE PHENOTYPE IN MICE

Purpose: The superoxide anion is implicated in numerous pathological processes and diseases such as inflammation, aging, cancer, and heart disease through oxidative damage of proteins, DNA, and lipids. The main antioxidant enzyme present in the extracellular space to protect against the harmful affects of superoxide is extracellular superoxide dismutase (ecSOD). Our lab previously discovered a novel allele for ecSOD in 129P3/J (129) mice termed the short allele distinct from the wild-type allele, found in most other strains, by two point mutations in the coding region and a 10 bp deletion in the 5□ UTR. The objective of our study is to examine the effect of the allelic differences on the ecSOD phenotype in various tissues.

Methods: Various tissues from 129 and C57 mice were harvested before and after heparin administration to examine tissue levels and distribution of ecSOD. Tissues were homogenized, and ecSOD activity and mass were measured by activity assay and by western blot, respectively. Tissue distribution was also exmined by immunohistochemistry.

Results: Lungs contain the highest levels of ecSOD followed by plasma, liver, and kidney, accounting for nearly 90% of the total ecSOD activity in both strains. Tissue ecSOD mass is higher in the liver, kidney, and plasma of the 129 strain when compared to C57, while lung levels are similar. Heparin administration results in an over 100% and 68% higher release of ecSOD from the liver and kidney, respectively of the 129 mice when compared to C57 mice. The lung, a tissue that contains the highest levels of ecSOD is not a source of heparin-releasable enzyme in either strain. Immunohistochemistry supported these observations, and showed differences in the ecSOD levels and distribution between strains in the liver and kidney. We also noted that as mice age, circulating ecSOD levels decline in 129 mice more rapidly when compared to those of C57.

Conclusions: Differences in ecSOD genotype have a significant effect on ecSOD phenotype, as reflected by tissue enzyme levels and the amount of heparin-releasable enzyme from the vasculature. This is likely the result of allele-specific differences in ecSOD synthesis, intracellular processing and tissue distribution.

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REPEATED ARTERIAL OCCLUSIONS IMPROVE VAGAL TRANSMISSION IN THE SINOATRIAL NODE WITHOUT ALTERING THE VAGOLYTIC RESPONSE TO OPIOIDS.

Purpose: To test the hypothesis that preconditioning like arterial occlusions of the sinoatrial (SA) node gradually improve vagal transmission and the resulting bradycardia, by abolishing the opposing vagolytic effect of the endogenous opioid, enkephalin.

Previous studies in this laboratory demonstrated that opioids (enkephalins) modify vagal transmission in the SA node in a bimodal manner. Enkephalins primarily interact with subtypes of the delta opioid receptor. Ultra-low doses of met-enkeph-arg-phe (MEAP) improve vagal transmission (vagotonic) and bradycardia by stimulating ´1-opioid receptors within the SA node. Higher doses of MEAP acting on ´-2opioid receptors interrupt vagal transmission (vagolytic) and reduce vagal bradycardia. Opioids have been implicated in the cardioprotective effect of ischemic preconditioning. Opioids mimic preconditioning by interaction with ´1-opioid receptors and opioid antagonists prevent ischemic preconditioning. Preconditioning of the SA node produced a vagotonic response during prolonged ischemia and the ´1-opioid receptor antagonist BNTX reversed the effect. The study that follows attempts to determine whether preconditioning or simply the prolonged ischemia are responsible for the observed vagotonic effect. The study will also test whether MEAP is vagotonic during ischemia because competing vagolytic effects are abolished.

Methods: A microdialysis probe was placed in the interstitium of the canine SA node and perfused with saline at 5¼l/min. The SA node artery was identified as a branch of the right coronary artery and traced visually to the SA node. A suture was placed around the artery near its origin and is occluded by a slipknot. The artery was occluded and released five times at 10-minute intervals. Vagally mediated bradycardia was tested at selected times during the preconditioning protocol and subsequent occlusions. The effects of added MEAP are evaluated after preconditioning and during the arterial occlusion.

Results: The preliminary data obtained suggests there is a gradual improvement in vagal transmission during the preconditioning protocol. However higher doses of MEAP (0.2nmol/min) administered by nodal microdialysis during arterial occlusion were clearly vagolytic.

Conclusions: These observations suggest that the vagotonic result observed during arterial occlusion requires preconditioning for complete expression and is not the result of abolishing competing vagolytic effects.

Sponsor: *Grant from Texas ARP program*

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VAGOTONIC EFFECTS OF ENKEPHALIN ARE NOT MEDIATED BY SYMPATHOLYTIC MECHANISMS

Purpose: Leucine-Enkephalin (LE) administered into the canine sinoatrial (SA) node by microdialysis reduced tachycardia during electrical stimulation of the cardiac sympathetic nerves (ansa subclavia). Ultra-low doses of methionine-enkephalin-arginine-phenylalanine (MEAP) similarly administered improved vagal transmission (vagotonic) during stimulation of the cervical vagus nerve and exaggerated the subsequent bradycardia. This study was designed to test the hypothesis that the vagotonic influence of MEAP is the result of reduced tachycardia secondary to a coincident sympatholytic effect.

Methods: Microdialysis probes were placed in the sinoatrial node of anesthetized mongrel dogs and infused with increasing doses of MEAP (5x10-14 - 1.5x10-9 moles/min). Adrenergic and cholinergic transmission was tested by recording heart rate and stimulating the ansa subclavia and cervical vagus nerves respectively. The right vagus nerve was stimulated at low (1-2 Hz) and high (3-4 Hz) frequencies for 15 sec with 2 minutes for recovery between the stimulations. Sympathetically mediated tachycardia was tested by stimulating the cardiac sympathetic nerves (45 sec at 1-2 Hz) at each dose.

Results: MEAP generally enhanced vagal bradycardia at doses near 5 x 10-13 moles/min and then gradually interrupted vagal function producing a clear vagolytic effect between 5x10-10 and 5x10-9 moles/min.. Sympathetic stimulations during vehicle administration increased heart rate by a mean of 35 +/- 10.6 bpm and the subsequent responses were not altered by increasing doses of MEAP.

Conclusions: Since MEAP has no apparent influence on sympathetic transmission, the sympatholytic withdrawal of competing tachycardia cannot explain the improved vagal transmission observed during the application of ultra-low doses of MEAP. The failure of MEAP to produce a sympatholytic effect similar to that observed for LE suggests that MEAP and LE use different receptors and/or MEAP is more selective than LE. Since the effect of LE was reversed by the k-antagonist, norbinaltorphimine, the current observations are consistent with the hypothesis that the sympatholytic opioid receptors are k-receptors. Additional dose response studies with selective k-agonists (dynorphin, U500488) will be required to verify the k-receptor character of the sympatholytic response.

Sponsor: *Tex-ARP Program*

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HYPEROXIA REVERSES SYMPATHOEXCITATORY STATE INDUCED BY INTERMITTENT HYPOXIC APNEA

Purpose: Obstructive sleep apnea (OSA) is associated with sustained elevation of basal muscle sympathetic nerve activity (MSNA). Morgan et al. showed that following 20 minutes of intermittent hypercapnic hypoxia, MSNA remains elevated for at least 20 min (J Appl Physiol,79:205, 1995). Recently, we showed that basal MSNA remains elevated following 20 min of intermittent hypoxic apnea (IHA) through 165 minutes of recovery, $p<0.01$ (Circulation 106:II-294, 2002). In addition, numerous studies have shown that MSNA is significantly reduced with hyperoxia in OSA patients. Therefore, we hypothesized that 20 min of IHA followed by 15 minutes of hyperoxia (100% O2) would attenuate the elevation in MSNA evoked by IHA.

Methods: Baseline MSNA (microneurography) and arterial pressure were recorded in 4 healthy normotensive subjects for 3 hrs of recovery from 20 min of IHA followed by 15 min of hyperoxia. During IHA, subjects were primed with 1-2 breaths of hypoxic gas prior to performing a 20 sec voluntary end-expiratory apnea: apneas were repeated once per minute for 20 min. Following two minutes of room air, subjects then breathed 100% O2 for 15 min.

Results: A significant main effect for basal MSNA over time was observed ($p<0.001$). Basal MSNA was elevated following IHA (122+6% of baseline, $p<0.01$) and returned to below baseline following hyperoxia (85+7% of baseline, $p=0.02$). Basal MSNA remained equal to or below baseline for 3 hours of recovery (104+8% of baseline at 180 min of recovery, $p=0.22$).

Conclusions: These data support the hypothesis that hyperoxia reverses the elevation of basal MSNA induced by intermittent hypoxic apnea. Furthermore, these data demonstrate that this effect is sustained for hours after IHA exposure and further support the role of hypoxia in mediating the chronic elevation of MSNA in OSA patients.

Sponsor:

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SYMPATHETIC RESPONSE TO PAIN USING THE COLD PRESSOR MODEL

Purpose: The purpose of this study is to develop a model for the quantitative relationship between acute pain stimulus, pain perception, & sympathetic nerve activity (SNA) in healthy individuals, testing the hypothesis that: (1) increased pain perception provokes graded increases in SNA; & (2) these SNA increases occur only above a pain threshold for SNA activation. This investigation will help guide & develop parallel & future studies in autonomic physiology of pain states in various diseases, the role of pain in maintaining such conditions, & potential benefits of Osteopathic Manipulative Therapy in reducing pain & normalizing autonomic tone in disease. In particular, these findings may be relevant to patients in whom presence of autonomic dysfunction is known to exacerbate disease & can potentially accelerate its progression.

Methods: 40 healthy normotensive volunteers aged 18-60 years are being recruited for this study in which 20 are r&omly assigned to 1 of 2 groups: cold pressor (CP) & time control (TC) groups. Subjects are studied at the same time of day & instrumented to record MSNA, arterial pressure, autonomic tone (spectral analysis of HR & BP variability), respiratory cycles, leg blood flow, & vascular resistances. After a 20-min. rest period, subjects enrolled in the CP group will submerse one h& for 2-min. in water baths set at 1 of 4 r&omly ordered temperatures (2, 5, 10, 15□C) with 20-min. to rest between stimuli. Control subjects will place one h& in a time-controlled water bath set at 25□C. Objective pain perception will be recorded using Borg□s 15-point Rating of Perceived Pain Scale just prior to each stimulus & 15 seconds before its termination. Data will be recorded continuously for 2-min. pre stimulus (baseline), 2-min. during stimulus, & 4-min. post stimulus (recovery).

Results: Recruitment for this study is not yet complete & preliminary results have yet to be fully analyzed, but study observations are consistent with current literature in that SNA increases with acute CP stimulus & does return toward baseline with its corresponding effects on HR, BP, & blood flow. The relationship of CP stimulus intensity, perceived pain, & time on these dependent variables will be analyzed at the conclusion of this study.

Conclusions: There are as yet no conclusions to be drawn until the study is completed. The preliminary data suggest that the cold pressor temperatures used are beyond the threshold, thus, higher temperatures will be used to help define this value.

Sponsor:

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HYPOXIA ACCOUNTS FOR SUSTAINED ELEVATION OF SNA AND ALTERED CHEMOREFLEX CONTROL FOLLOWING INTERMITTENT APNEA.

Purpose: Recently, we demonstrated a sustained elevation of SNA and augmented chemoreflex sensitivity for at least 180 min and 165 min, respectively, following 20 min of intermittent hypoxic apneas (Circulation, 2002). However, the relative contribution of hypoxia, hypercapnia and absent ventilation on this response is unknown and was the focus of this study.

Methods: 21 subjects were randomly assigned to one of three groups (hypoxic apnea, hypercapnic hypoxia, and isocapnic hypoxia). Subjects were exposed to 30 s of the perturbation every min for 20 min. SNA (microneurography), arterial pressure (Finapres) and heart rate (ECG) were measured continuously during baseline, the perturbation and for 3 hr of room air recovery. In addition, chemoreflex control of SNA was assessed during baseline and every 15 min throughout recovery by the SNA response to a single voluntary apnea. Each recovery apnea was matched to a baseline apnea with a similar nadir of SaO2.

Results: Total SNA was elevated compared to baseline following treatment and remained elevated throughout the 3 hr recovery period ($p<0.05$). The increased SNA was not different between the three treatment groups ($p=0.50$). A significant main effect for chemoreflex control of SNA over time was observed ($p<0.05$). The SNA response to a single apnea was augmented through 180 min of recovery ($p<0.05$) and was not different between the 3 treatment groups ($p=0.69$).

Conclusions: These data suggest that hypoxia is the primary mediator of the sustained elevation of SNA and augmented chemoreflex sensitivity following periods of intermittent hypoxic apneas.

Sponsor:

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APOLIPOPROTEIN E SECRETION FROM J774, A MURINE MACROPHAGE CELL LINE STABLY TRANSFECTED WITH HUMAN APOE2, E3 AND E4 ISOFORMS..

Purpose: Apolipoprotein E (apoE) is a protein component of plasma and interstitial fluid lipoproteins and is synthesized by a number of tissues including the liver, brain, skin and tissue macrophages. ApoE functions as a ligand for receptor mediated cholesterol uptake by the LDL receptor in the reverse cholesterol transport pathway, in the localized transport of lipids and in modulating the immune response by T-lymphocytes. ApoE has three alleles of which apoE3 is the most common. The three isoforms apoE2, apoE3, apoE4 differ by a single amino acid substitution and appear to differ in various aspects of their metabolism. I propose to study these differences.

Methods: Our laboratory has previously made stable clones of J774, a murine macrophage cell line, transfected with human apoE2, apoE3, apoE4. Currently, I am in the process of evaluating the expression of apoE from these cells. Briefly, cells are grown until confluent with 400ug/ml geniticin. Cells are then incubated with DMEM + 10% FBS in the absence of geniticin for 24h. Fresh medium without FBS and drug is added and apoE secretion into medium is monitored at 0, 2, 4, 8 and 24-hours. The expression of hapoE is measured by western blotting.

Results: ApoE secretion by J774/E2 and J774/E3 is detected within 2 hours of incubation. ApoE secretion by J774/E4 cells is only measurable at 24 hours. ApoE secretion is the highest in the J774/E3 cells followed by the J774/E2 cells and finally the J774/E4 cells.

Conclusions: The three clones secrete apoE into the medium at different rates and apoE can be detected within 2 hours. These cells will therefore provide a valuable tool to examine allele-specific aspects of intracellular apoE processing, its role in cholesterol efflux and potential interaction with caveolins.

Sponsor:

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TGF-BETA 1 STIMULATES SMOOTH MUSCLE CELL DIFFERENTATION THROUGH THE ACTIVATION OF PKN: A ROLE FOR P38 MAP KINASE SIGNALING

Purpose: Differentiated vascular smooth muscle cells (SMCs) exhibit a work phenotype characterized by expression of several well-documented contractile apparatus-associated proteins. However, when exposed to mitogens such as serum or growth factors, SMCs retain the ability to de-differentiate into an □synthetic□ proliferative phenotype, in which they lack contractile myofilaments. Proliferation of SMCs is involved in the formation of atherosclerotic plaques as well as arterial restenosis following balloon angioplasty. Understanding the mechanisms involved in maintaining SMC differentiation process is critical to the development of therapies and treatments for the abnormal growth seen in these disease states. In this study, we examined the existence of a novel signaling pathway whereby transforming growth factor-beta 1 (TGF-beta 1) activates the protein kinase C-related kinase PKN to induce differentiation of SMCs.

Methods: PAC-1 SMCs were cultured in the absence or presence of 2.5ng/mL TGF-beta 1. For transient transfection experiments, PAC-1 cells were transfected with empty vector plasmid, constitutively active PKN (PKN-AF3) or kinase dead PKN (K644E).

Results: First, we demonstrated that TGF-beta 1 induced differentiation of PAC-1 cells through actin re-organization and up-regulation of several SMC marker genes, including smooth muscle alpha actin (SM alpha-actin), smooth muscle myosin heavy chain (SM-MHC) and SM22. TGF-beta 1 induced differentiation was correlated with a reduction in cell proliferation as well as increased intracellular activity of RhoA and its downstream target, PKN. Over-expression of active PKN was sufficient to increase the transcriptional activity of the SM alpha-actin, SM-MHC and SM22 promoters in PAC-1 cells. In addition, the activity of three transcription factors (SRF, GATA and MEF2), which are known to regulate expression of SMC marker genes, was also increased. Finally, examination of MAPK signaling cascades demonstrated that TGF-beta 1 stimulation of PAC-1 cells resulted in increased activity of MKK3/6 and p38 MAPK with decreased activity of ERK1/2 and JNK1/2. Co-expression of dominant negative p38 MAPK was sufficient to abolish PKN-mediated activation of SRF, GATA and MEF2 as well as PKN-mediated activation of SMC marker gene promoters.

Conclusions: Taken together, these results identify components of an important intracellular signaling pathway through which PKN promotes differentiation of SMCs by up-regulating expression of SMC marker genes.

Sponsor: *NIH, AHA*

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DEVELOPMENT OF A METHOD TO QUANTIFY GM-1 IN SINOATRIAL NODE

Purpose: Opioids function as neuromodulators by moderating neurotransmitter release. Crain and Shen proposed that excitatory opioid receptors increase their own activity through a positive feedback loop. Ultra-low opioid concentrations stimulate delta-1-opioid receptors and activate adenylyl cyclase. The resulting increase in the cyclic-AMP dependent protein kinase, phosphorylates glycosyltransferase, and increases the synthesis of GM-1. This increase in GM-1 theoretically improves the efficiency of excitatory opioid receptor coupling.
Cholera toxin ADP-ribosylates Gs-alpha by specifically binding to the membrane ganglioside GM-1 through a □two-fingered grip□ involving the terminal galactose and sialic acid moieties of GM-1. The observation that cholera toxin-B (CTX-B) specifically binds GM-1 suggested that labeled CTX-B might be used to quantify GM-1 in picomolar concentrations.

Methods: 1) Gangliosides will be extracted from 2-4 g of canine sinoatrial (SA) node/atrial tissue with 10-15 volumes of chloroform-methanol. Particulates will be filtered and rinsed twice with 10 volumes of same solvent. The chloroform-methanol will be evaporated and the residue redissolved in 95% ethanol. Aliquots (50 ul) of the extract and authentic standards (0.75 - 6.0 pmoles) will be distributed into polystyrene microwells and the GM-1 will be allowed to adsorb for 100 min at room temperature. The wells will be rinsed three times with PBS and incubated with added FITC-labeled CTX-B in the dark. The free CTX-B will be washed off and the bound ligand will be quantified by spectrofluorometry.
2) Canine SA node/atrial tissue will be homogenized in buffered saline and the synaptosomal membranes will be collected by differential centrifugation. The membranes will be resuspended and washed twice with buffer. The membranes will be incubated with FITC-labeled CTX-B. After incubation the bound and free constituents will be separated by centrifugation and the bound ligand will be quantified by spectrofluorometry and expressed as relative fluorescence per mg protein.

Results: Once validated these methods will be employed to test the hypothesis that extended stimulation of delta-1-receptors in the SA node increases synthesis of the neural membrane ganglioside, GM-1.

Conclusions: These studies should add significantly to our understanding of paracrine influences on the neural control of cardiac rhythms

Sponsor: *Texas ARP*

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TGFBETA1 DELAYS G2/M PHASE PROGRESSION VIA PKN SIGNALING IN VASCULAR SMOOTH MUSCLE CELLS

Purpose: Mature vascular smooth muscle cells (VSMC) are unique in that they can switch back and forth between proliferative and differentiated phenotypes. Aberrant proliferation of VSMC reappears with the onset of atherosclerosis and is a characteristic of restenosis following balloon angioplasty. A better understanding of VSMC cell cycle regulation is critical for developing therapies to prevent or to lessen the occurrence of these diseases.

Methods: Transforming growth factor-b1 (TGF-b1) is known to inhibit VSMC cell cycle progression; however, the signaling pathways through which this is accomplished are not completely understood. In this study, we demonstrate that TGF-b1 delays G2/M phase progression in part via activating PKN, a protein kinase C-related kinase, in VSMCs synchronized at G1/S phase border.

Results: We show that treatment of actively proliferating PAC-1 cells (a rat pulmonary VSMC line) with TGF-b1 inhibits proliferation and induces the cells to adopt a differentiated phenotype. Flow cytometry analysis reveals that pre-treatment with PKN inhibitors abolishes TGF-b1-induced delay of the cell cycle progression. Moreover, activation of PKN temporally correlates with the timing of G2/M phase progression. In dividing smooth muscle cells like all higher eukaryotic cells, entry into mitosis is governed by Cdc2/cyclin B complex, which is activated by the phosphatase Cdc25C at onset of mitosis. Immunocytochemistry studies demonstrate that PKN and Cdc25C co-localize only in the nuclei of dividing (M phase) cells but not in the interphase cells. Additionally, Cdc25C is co-immunoprecipitated with active PKN but not with kinase-dead PKN.

Conclusions: Taken together, these data suggest that PKN inhibits G2/M phase progression by directly binding to Cdc25C and inhibiting its activity. Augmenting PKN-Cdc25C-Cdc2 signaling may provide a potential therapeutic approach to counter abnormal VSMC proliferation in restenotic lesions.

Sponsor:

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ANALYSIS OF THE ROLE OF PKN AND RHO IN SMOOTH MUSCLE CELL PROLIFERATION AND DIFFERETIATION: AN INDUCIBLE TRANSGENIC MOUSE MODEL

Purpose: A novel serine/threonine protein kinase, designated PKN, has a catalytic domain homologous to the PKC family and unique amino-terminal sequences. PKN plays a significant role in the control of mitotic timing by inhibition of Cdc25C and functions as a cell-cycle regulator. Rho, a small GTP-binding protein, binds to PKN in a GTP-dependent fashion leading to the activation of PKN. Rho regulates actin-based cytoskeletal structures, including focal adhesions, stress fibers, the contractile ring, and also works as a switch in stimulus-evoked cell adhesion and cytokinesis. In this study, we seek to identify in vivo functions of PKN and Rho GTPAse in smooth muscle cells.

Methods: We will utilize the benefits of an optimized tet-off system and a specific smooth muscle gene (SM22) promoter to generate a smooth muscle-specific, doxycycline (Dox) controlled over-expression system in transgenic mice. A DNA construct will be generated in which the codon optimized reverse tetracycline transactivator (rtTA) is placed under control of a smooth muscle-specific SM22 promoter. Transgenic mice containing this construct will expresse rtTA exclusively in smooth muscle cells. These mice will be crossed to a second transgenic line containing a bi-directional promoter centered on a tet responsive element driving either tagged PKN gene or tagged Rho gene. Dox dependent smooth muscle-specific induction of the tagged PKN and Rho proteins will be demonstrated by western blot and immunocytochemical analysis.

Results: We hypothesize that the generation of this inducible transgenic mouse model will provide a tightly regulated over expression system in adult smooth muscle. This will allow us to study the function of PKN and Rho GTPase in regulation of smooth muscle cell proliferation and differentiation.

Conclusions: The application of this type of system will prove invaluable to giving insights into the function and regulation of PKN and Rho under physiological and pathological conditions.

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A METHOLOGIC APPROACH FOR EVALUATING VAGAL TRANSMISSION AND ACETYLCHOLINE RELEASE IN THE SA NODE

Purpose: Delta opioid receptors in the sinoatrial (SA) node exert dual effects on vagal bradychardia. Low doses of the native delta-agonist, met-enkephalin-arg-phe (MEAP) improve vagal bradychardia by stimulating delta-1 receptors. In contrast, higher-dose MEAP interrupts vagal transmission by interaction with delta-2 receptors. The following method is being developed to test the hypothesis that MEAP modulates vagal transmission by modifying the release of acetylcholine within the SA node.

Methods: The canine right atrium is isolated, and the right coronary artery is cannulated to provide vascular access to the SA node. The artery is perfused at constant pressure with Hepes buffered Krebs-Heinselet solution (pH 7). Hemicholinium-3, is added to prevent choline re-uptake by vesicular choline transport. Perfusate is collected before, during, and after vagal stimulation. After each experiment the perfused territory is marked with Evans Blue dye, excised and weighed. Recovered acetylcholine is hydrolyzed by endogenous acetylcholine esterase and converted to betaine and H2O2 with added choline oxidase. Horseradish peroxidase, then stochiometrically oxidizes Amplex Red to its fluorescent product resorufin, and is quantified by spectrofluorometry (Abs/Em: 563/587). The choline and acetylcholine will be independently verified by thin layer chromatoghaphy.

Results: Initial experiments indicate that the method sensitively detects nanomoles of choline per gram of tissue, and right vagal stimulation produces a clear increase in choline overflow from the SA node area.

Conclusions: This method promises to be a sensitive tool for evaluating the role of opioids in the paracrine regulation of acetylcholine release.

Sponsor: *NIGMS and Texas ARP*

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CARDIAC FUNCTION AND PHOSPHORYLATION POTENTIAL DURING CARDIAC RESUSCITATION: PYRUVATE THERAPY

Purpose: The impacts of cardiac massage and exogenous pyruvate on energy state of arrested myocardium and post-arrest contractile recovery were determined in open-chest dogs

Methods: Five min after pacing-induced ventricular fibrillation, cardiac massage was delivered for 5 min to increase aortic pressure to ~70 mm Hg, then epicardial DC countershocks (5-10 J) were applied to restore sinus rhythm. Pyruvate (Pyr) was infused via femoral vein throughout massage and the first 25 min recovery (ROSC) to raise plasma pyruvate to 3.5-4 mM. Left ventricular maximum dP/dt indexed cardiac function. Phosphocreatine phosphorylation potential (~PCr: [PCr]/([Cr][Pi])) was measured in snap-frozen left ventricular biopsies.

Results: In non-pyruvate control experiments, dP/dt (mm Hg/sec) fell from pre-arrest 3100 +/- 180 (means +/- SEM, n = 6-10) to 2410 +/- 170 at 25 min ROSC. Pyruvate increased dP/dt ($P < 0.05$) to 3290 +/- 260 at 25 min ROSC. At 180 min ROSC, dP/dt remained somewhat higher in the pyruvate group (3000 +/- 180 vs. 2340 +/- 240) even though pyruvate infusion was dicontinued at 25 min. ~PCr (1/M) fell from 174 +/- 19 to 3 +/- 1 at 5 min arrest. Cardiac massage partially restored ~PCr to 30 +/- 6. ~PCr recovered to 176 +/- 31 and 186 +/- 25 at 25 and 180 min ROSC. Pyruvate enhanced ~PCr during massage (63 +/- 15); at 25 min ROSC, ~PCr in pyruvate-treated myocardium (238 +/- 24) was at or above the control level, despite higher contractile function and energy demand.

Conclusions: Pyruvate is an effective adjunct to cardiac compression as a means of restoring myocardial energy reserves and improving post-arrest cardiac function. (NHLBI 71684)

Sponsor: *NHLBI*

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THE REPRODUCIBILITY OF THE ACETYLENE RE-BREATE METHOD FOR DETERMINING CARDIAC OUTPUT OF HUMANS DURING EXERCISE

Purpose: We sought to establish the in-laboratory reproducibility of the foreign gas re-breathe method using acetylene in the measurement of cardiac output (Q) during exercise in humans.

Methods: 13 healthy volunteers (8 men and 5 women) with an average age = 23.9 + 5.9 yrs., height = 165.9 + 32.5 cm, weight = 75.9 + 15.1 kg were recruited and provided written informed consent to act as subjects. Each subject performed a progressive workload bicycle exercise test to maximal effort on an electrically braked bicycle ergometer (Scifit 5500). The determination of maximal oxygen uptake (VO2MAX) was obtained from respiratory gases and ventilation volumes. The group average VO2MAX was 35.2 + 7.6 ml O2/kg/min. On two separate days, each subject performed two randomly assigned sub-maximal (40 % and 60% of VO2MAX) exercise tests for 30 minutes. During each sub-maximal exercise, measures of Q and VO2 were made at 5 minute increments, after an allotted ten minutes to reach steady state.

Results: Measures of Q were not different between each time interval ($P>0.05$) at any given workload. The correlation coefficients of reproducibility for Q (r) were 0.88 and 0.87 for the 40% VO2MAX and 60% VO2MAX exercise trials, respectively. The average coefficient of variation for Q measures during both sub-maximal workload exercises during both days was 7.8% (ranging from 6.5% to 9.4%).

Conclusions: These findings indicate that the in-laboratory measurements of Q during sub-maximal exercise can be performed with an acceptable error of measurement and reproducibility.

Sponsor:

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PYRUVATE-ENHANCED CARDIOPLEGIA ATTENUATES OXIDATIVE STRESS DURING CARDIOPLUMONARY BYPASS

Purpose: Cardiopulmonary bypass (CPB) elicits a systemic inflammatory response characterized by activation of inflammatory cells, increased production of cytokines, and formation of oxygen free radicals. We recently demonstrated that cardioplegia fortified with pyruvate, an energy yielding fuel and antioxidant, minimized myocardial injury and hastened post-surgical recovery of cardiac function in CPB patients, relative to the standard lactate-based cardioplegia. The objective of this study was to identify the mechanisms of improvements seen in these patients.

Methods: Anesthetized pigs were subjected to 60 min of CPB with 40 min ischemic cross clamp, and then allowed to recover for four hours. Pigs were administered either standard lactate-based cardioplegia or pyruvate-enhanced cardioplegia in 4 vol blood. Sham control pigs were instrumented with the bypass cannulae without performing CPB. 8-Isoprostane, an indicator of oxidative stress, and C-reactive protein (CRP), an acute phase reactant were measured in plasma samples taken before bypass and at thirty min intervals during and following CPB. Tissue samples from the lungs and heart taken at the termination of the experiment were fixed, mounted and stained, and examined for the presence of neutrophil infiltration.

Results: CPB with lactate-based cardioplegia caused a substantial increase in oxidative stress, exemplified by an eight-fold maximal increase in 8-isoprostane at 30 min of bypass. Pyruvate-enhanced cardioplegia lowered the increase in 8-isoprostane to the two-fold increase seen in the sham control pigs. Pyruvate cardioplegia also appeared to decrease pulmonary neutrophil infiltration as compared to the sham control experiments. CRP varied throughout the experiments but tended to be lower at the end of recovery in pigs arrested with pyruvate cardioplegia versus those arrested with lactate cardioplegia.

Conclusions: Pyruvate-enhanced cardioplegia appears to mitigate the oxidative stress and inflammatory response associated with CPB. These findings may at least partially explain the improved recovery of patients receiving pyruvate-fortified cardioplegia during CPB.

Sponsor: *Osteopathic Heritage Foundation*

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PURIFICATION OF MOUSE ECSOD

Purpose: Extracellular superoxide dismutase (ecSOD), an antioxidant enzyme found in the extracellular matrix of tissues, is a major regulator of reactive oxygen species (ROS)-mediated tissue damage. The purpose of this study was to develop a method to isolate and purify ecSOD from mouse plasma in order to determine the sequence of this protein. This information will help us to investigate the difference in specific activity of enzyme between the wild type and a novel ecSOD allele previously isolated by our lab.

Methods: A general purification scheme has been adopted, which involves isolation of plasma from heparin-injected SW mice. The pooled plasma is fractionated by FPLC and the fractions are dialyzed against 250mM NaCl in 50mM HEPES. Further purification is obtained by concanavalin A-sepharose chromatography and the glycoproteins are eluted with a-methylmannoside. The sample is then applied to an immunoaffinity column for final purification. Total SOD activity will be monitored by an activity assay and ecSOD mass by western blot..

Results: Preliminary data indicate that this approach will be successful since application of the plasma to FPLC results in the separation of ecSOD from contaminants of other molecular weight. Con A-sepharose separates ecSOD from the other isoforms of SOD (CuZnSOD and MnSOD) since con A binds to glycosylated proteins, which in this case is a property unique to ecSOD. The purity of the enzyme has increased several fold as determined by western blot. Immunoaffinity chromatography will allow for further purification of the enzyme as a result of binding to the ecSOD antibody.1

Conclusions: Western blotting and silver staining have revealed that a semi-pure sample was obtained thus far that had significantly less contamination from other proteins. The purified ecSOD sample obtained from this study will be sequenced. The successful completion of this project will enable us to begin the elucidation of observed differences in the ecSOD phenotype in mice differing in their genotype.

Sponsor:

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YING YANG-1 (YY1) DEFINES THE ACTIVATION THRESHOLD FOR SMOOTH MUSCLE MYOSIN HEAVY CHAIN PROMOTER ACTIVITY

Purpose: Physical competition between transcriptional enhancers such as GATA, SRF, C/EBPb and CTF/NF1 and the transcriptional repressor YY1 appears important to gene regulation in vascular myocytes. 2.5kb of the proximal promoter sequence of Smooth Muscle Myosin Heavy Chain (SMM-HC) encodes twenty-four binding sites for YY1 and multiple sites for the previously mentioned enhancers. Domains where YY1 sites appose or overlap enhancers sites are configured to allow competitive factor binding. We refer to these domains as Dual Regulatory Domains (DRD).

Methods: Using three serial truncations of the SMM-HC promoter we have isolated the effect of specific DRDs containing C/EBPb (-1454bp and -637bp) that potentially compete with YY1 (-1463bp, and -648bp). In the pulmonary arterial myocyte cell line PAC-1, we tested the competitive influence between enhancers and YY1 using three concurrent measurements; i) transcription factor maximal activity by increasing gene dose; ii) YY11/2max repressive dose; and iii) % recovery of maximal activity by increasing enhancer in the presence of YY11/2max repressive dose.

Results: Predictably, YY1 dominantly repressed the promoter activity of each SMM-HC promoter truncation. Moreover, C/EBPb demonstrated a dose-dependent augmentation in promoter activity for all promoter truncations. While the effective dose of C/EBPb remained relatively constant, the % recovery of maximal activity was inversely proportional to the quantity of enhancer-specific DRDs. In the context of C/EBPb, p1621 contains 2 DRD and restored roughly 33% of C/EBPb activity. p1249 contains 1 DRD and restored roughly 70% of C/EBPb activity. p602 contains no DRD and was capable of restoring 100% of C/EBPb activity. Finally, titrating increasing YY1 against 1/2max activating dose of C/EBPb demonstrated proportional reliance on DRD in terms restoring 100% repression. Moreover, mutation of both DRD both singularly and collectively uncoupled the competitive regulatory influence witnessed between C/EBPb and YY1 mimicking proportional response to wild-type promoter of fewer DRD.

Conclusions: These data argue strongly that SMM-HC promoter activity is directly regimented by multiple points of YY1 repression, defining this promoters activation threshold, and alteration to this promoters activity require overriding YY1 repression events.

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ACUTE CARDIAC ARREST INACTIVATES MYOCARDIAL METABOLIC ENZYMES

Purpose: During cardiac arrest, global myocardial ischemia elicits oxidative stress which can deplete antioxidant defenses and inactivate proteins within cardiac myocytes. Pyruvate, a powerful metabolic antioxidant, bolsters the glutathione antioxidant system in myocardium. We tested the hypotheses that cardiac arrest depletes endogenous antioxidants and inactivates myocardial enzymes, and that pyruvate reactivates these enzymes by restoring antioxidant redox state.

Methods: Open-chest dogs were subjected to 5 min cardiac arrest, 5 min cardiac massage, and defibrillation by DC countershocks. Pyruvate was infused iv throughout massage and 25 min recovery (ROSC) to maintain its arterial plasma concentration at ~3.5 mM. Glutathione redox state, i.e. the ratio of glutathione (GSH) to glutathione disulfide (GSSG), and activities of the key enzymes creatine kinase (CK), phosphofructokinase (PFK) and glucose 6-phosphate dehydrogenase (G6PDH) were measured in snap-frozen biopsies of left ventricular myocardium.

Results: The oxidative stress of cardiac arrest partially inactivates key metabolic enzymes, but the enzymes spontaneously recover with GSH redox.state following defibrillation. Pyruvate bolstered GSH/GSSG post-arrest but did not further activate the enzymes.

	Sham	Arrest	ROSC No Tx	ROSC Pyr
GSH/GSSG	20 ± 1.8	9.3 ± 0.9*	20 ± 2.7	32 ± 2.9*
CK	37 ± 3.0	25 ± 1.3*	28 ± 3.7	33 ± 4.2
PFK	0.74±0.07	0.38±0.04*	0.47±0.06	0.59±0.10
G6PDH	7.0 ± 0.8	3.0 ± 0.5*	6.1 ± 1.2	5.5 ± 1.0

Means ± SE, n=6; * P<0.05 v sham, P<0.05 v no treatment (NoTx). CK, PFK: U/mg protein; G6PDH: mU/mg protein

Conclusions: Spontaneous post-arrest recovery of cardiac enzymes is not enhanced by pyruvate despite increased GSH redox state.

Sponsor: *NIH support: HL 76184*

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CHARACTERIZATION OF RECOMBINANT LECITHIN:CHOLESTEROL ACYLTRANSFERASE, SECRETED BY PICHIA PASTORIS YEAST CELLS.

Purpose: Even though Purified LCAT has bee n available over 25 years and the primary structure of LCAT as well as the essential features of the catalytic site has been determined. The three-dimensional structure of the enzyme remains to be elucidated due to the lack of diffraction-grade crystals for X-ray analysis. Therefore, a Pichia pastoris expression system has been developed to provide in the future sufficient amount of enzyme for x-ray crystallography and multi-dimensional NMR analysis of the 15N, 13C and 2H labeled enzyme. These proposed studies are aimed to solve the solution structure and subsequently the complete three-dimensional structure of LCAT and highlight the details of its salient catalytic features.

Methods: A. Plasmid Vector Construct
Pichia pastoris yeast expression system consists of human LCAT cDNA cloned into pPICZ±A vector along with a removable polyhistidine tag at the amino-terminal of LCAT. (Dr. Min Lee at University of British Columbia, Vancouver, British Columbia, Canada has provided the vector construct).
B. Transformation of Pichia pastoris
The EasyCompTM (Invitrogen) transformation method will be used to transform the Pichia pastoris yeast cells. Transformants will be selected on agar plate containing Zeocine (100 mg/ml) as antibiotic. Further screening will done by polymerase chain reaction (PCR) and by reverse transcription polymerase chain reaction (RT-PCR) for the correct integration.

Results: Pichia pastoris GS115 (his4) strain has been transformed by LCAT cDNA cloned into pPICZ±A vector along with a removable polyhistidine tag at the amino-terminal of LCAT. Transformants have been selected on agar plate containing zeocine (100 mg/ml) as an antibiotic and screened by replica plating, polymerase chain reaction (PCR), and reverse transcriptase polymerase chain reaction (RT-PCR) to confirm the correct integration of the LCAT gene cDNA + pPICZaA vector into Pichia genome. The recombinant LCAT expressed by Yeast cells was purified by Talon TM resins (cobalt-based immobilized metal affinity chromatography resins (IMAC)), designed to purify recombinant polyhistidine-tagged proteins (bush et al., 1991).

Conclusions: The current work consists of recovering the activity of the recombinant LCAT (rLCAT) because LCAT produced by the yeast cells is not glycosylated the same way as the human plasma LCAT. The glycoprotein glycan chains in Pichia pastoris are shorter, with high mannose content. That difference in glycosylation affects the function of the LCAT.

Sponsor:

400

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CHANGES IN ORIENTATION OF ACTIN DURING CONTRACTION OF MUSCLE

Purpose: It is well documented that muscle contraction results from cyclic rotations of actin-bound myosin cross-bridges. The role of actin is hypothesized to be limited to accelerating phosphate release from myosin and to serving as a rigid substrate for cross-bridge rotations. In order to test this hypothesis, we have measured actin rotations during contraction of a skeletal muscle.

Methods: Actin filaments of rabbit psoas fiber were labeled with rhodamine-phalloidin. Muscle contraction was induced by a pulse of ATP photogenerated from caged precursor. ATP induced a single turnover of cross-bridges. The rotations were measured by anisotropy of fluorescence originating from a small volume defined by a narrow aperture of a confocal microscope.

Results: The anisotropy of phalloidin-actin changed rapidly at first and was followed by a slow relaxation to a steady-state value. The kinetics of orientation changes of actin and myosin were the same. Extracting myosin abolished anisotropy changes. To test whether the rotation of actin was imposed by cross-bridges or whether it reflected hydrolytic activity of actin itself, we labeled actin with fluorescent ADP. The time course of anisotropy change of fluorescent nucleotide was similar to that of phalloidin-actin.

Conclusions: These results suggest that orientation changes of actin are caused by dissociation and rebinding of myosin cross-bridges, and that during contraction nucleotide does not dissociate from actin.

Sponsor:

402

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NA,K-ATPASES OF NUCLEI OF THE RABBIT KIDNEY

Purpose: Compartmentalization via membranes is the key to cellular function and specialization. The membranes are relatively impermeable to ions and polar substances. Therefore, channels, that move substances downstream with their gradients, and pumps, that move substances upstream against their gradients, have evolved as intrinsic membrane proteins. Na,K-ATPase is a P-type ATPase of the plasma membrane that transports 3 Na+ out of the cell and 2 K+ into the cell. Since both ions are moving upstream against steep gradients, the energy is supplied by the hydrolysis of ATP. Recent studies of rat hepatocytes have demonstrated the presence of active Na,K-ATPases in the inner membrane of the nuclear envelope. The focus of our research has been to test for nuclear Na,K-ATPase in rabbit kidney.

Methods: Nuclei were isolated from the kidney by differential centrifugation followed by sucrose barrier centrifugation. The nuclear fraction was confirmed to be primarily nuclei by UV/Vis spectroscopy. To determine whether the nuclei had Na,K-ATPase, indirect immunofluorescence was performed with antibodies to the a1, a2 and a3 Na,K-ATPase catalytic subunit isoforms. DAPI was used as a counterstain for DNA.

Results: An A260/A280 ratio of 1.1 was obtained for the nuclear fraction. This shows that there was more DNA than protein in the fraction. There was little absorbance at 415 nm, the Soret band for heme, a component of cytochromes. The a1 and a2 isoform antisera stained the periphery of the nuclei, i.e. surrounded the DAPI-stained DNA. Staining with the a3-antibody was not observed.

Conclusions: There is Na,K-ATPase in renal nuclei. This is the third tissue with nuclear Na,K-ATPase. Future studies will define the function of the renal-nuclear Na,K-ATPases.

Sponsor: *none*

403

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OXIDATIVE DNA DAMAGE IN DNA REPAIR DEFICIENT MUTANTS OF SACCHAROMYCES CEREVISIAE..

Purpose: Oxidative DNA damage plays a major role in aging, cancer and other diseases such as Alzheimer. Our approach is to investigate if oxidative DNA damage can be responsible for the phosphorylation of Rad53 kinase, involved in checkpoint control and used here as an indicator for a cellular stress response to DNA damage.

Methods: To analyse the oxidative DNA damage response in DNA repair deficient strains, we used existing single mutant strains as well as double mutant strains. We also constructed novel double mutant combination using PCR-mediated gene transplacement. Rad53 was detected by Western Blotting and phosphorylation was identified as band retardation in a polyacrylamide gel.

Results: In certain DNA repair deficient mutants undergoing aerobic metabolism we found phosphorylation of checkpoint kinase Rad53 during extended incubation in stationary phase. In a rho zero strain i.e. without functional mitochondria, phosphorylation was absent. Phosphorylation was only detected if a combination of repair pathways was inactivated. These include nucleotide excision repair and base excision repair or nucleotide excision repair and non-homologous endjoining (represented by HDF1, encoding the yeast Ku protein homolog).

Conclusions: Phosphorylation of checkpoint kinase Rad53 in DNA repair deficient yeast occurs as a result of unrepaired DNA damage due to mitochondria-generated oxidative species. Nucleotide excision Repair (NER), base excision repair (BER) and a protein binding to double strand breaks (Ku) have overlapping functions in repairing such oxidative DNA damage. This also defines the novel role for yeast Ku protein in oxidative DNA damage protection.

Sponsor:

404

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ROLES OF ANDROGEN RECEPTOR IN GRANULOPOIESIS

Purpose: Neutrophils are major effector cells in the host defense response to microbial infection. In order to investigate the roles of androgen receptor (AR) in granulopoiesis, AR-knock out mice are used. Unexpectedly, we found that mice lacking the androgen receptor (ARKO) have significantly decreased numbers of neutrophils in both circulation and bone marrow. The objective of this study is to elucidate the role of androgen receptor in granulopoiesis.

Methods: Both peripheral blood and marrow cells from ARKO and control mice are obtained to analyze the following characteristics: 1) granulocytoc linage (Gr+) cells, 2) colony formation in methylcellulose by bone marrow cells, 3) treatment with androgen in castrated animals, 4) cell proliferation and differentiation, 5) cell cycle analysis and 6) bactericidal activity .

Results: The results have shown that Gr+ cell numbers are found reduced in the marrow of ARKO animals. In addition, androgen replacement is able to elevate low neutrophil levels in castrated mice but not ARKO mice. In response to GM-CSF or G-CSF in vitro, the frequency of colony forming cells from bone marrow populations derived from ARKO mice and wildtype controls are comparable. However, interestingly, cell cycle analysis and BrdU incorporation further suggest that cell expansion activity of granulocytic precursors is markedly reduced in ARKO mice. Furthermore, the differential counting of bone marrow cells demonstrates that in ARKO mice, the neutrophil differentiation is blocked between the promyelocyte and myelocyte stage. Moreover, ARKO mice are more susceptible to death caused by intraperitoneal Escherichia coli infection.

Conclusions: These data suggest that AR is needed for terminal differentiation in the normal neutrophil development. In addition, expression of secondary and tertiary granule components is decreased in ARKO neutrophils, leading to reduction of bactericidal activity. Together, our results indicate a novel unexpected role for AR in the regulation of neutrophil development and the host defense response to acute infection.

Sponsor:

405

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OSMOTIC CELL VOLUME CHANGES REGULATE ENOS ACTIVITY OF ENDOTHELIAL CELLS

Purpose: Endothelial cells of blood vessels can exposed to osmotic stress in tissues such as kidney medulla. We hypothesized that osmotic stress caused cell volume changes can regulate enodthelial eNOS activity and nitric oxide generation. Aim of the study is to explore how endothelial cells respond to osmotic stress with alteration of NO production and the mechanisms regulating NO production in endothelial cells.

Methods: Human umbilical vein endothelial cells (HUVEC) were used as an experimental model. Imuunofluoresent microscopy was used to visualize eNOS expression in HUVEC. To test eNOS activity of HUVEC, we used a NO-sensitive microelectrode system to detect [NO] released from HUVEC under superfusion. To test role of Ca++ signaling in endothelial cell response to osmotic challenges, we used confocal microscopy with Ca++-sensitive fluorescent dye, calcium-green for [Ca++]i on HUVEC. Western blotting was performed (with antibodies to eNOS, Akt-ser473, eNOS-ser1177) for estimating eNOS, Akt and eNOS phosphorylation of HUVEC under osmotic challenges.

Results: HUVEC have active eNOS, generate 50-60 nM NO in isotonic HBSS, which is L-arginine (100μM) dependent and inhibited by L-NAME (500μM). Cell shrinking (in HBSS+200mM mannitol) decreased NO production to 30-40nM that was recoverable by returning to isotonic HBSS. A fast [Ca++]i increase follows osmotic cell swelling but not cell shrinking. In Ca++-free HBSS, NO production decreased to 30-40 nM and 20-30 nM when cell shrinking. Further blocking [Ca++]i by BAPTA/AM lowers NO to 10nM range no matter cell swelling or shrinking. The cell swelling is associated with higher ratio of eNOS dimer/monomer, more phosphorylated Akt and eNOS than in shrinking cells. Inhibition of Akt signaling with wortmannin did not block cell swelling caused acute increase of NO production.

Conclusions: In conclusion, eNOS activity is regulated by osmotic cell volume changes, cell shrinking inhibits eNOS that can be reactivated during cell swelling. Intracellular calcium signaling and eNOS dimerization mediate eNOS activation during cell swelling. (Supported by American Heart Association and Faculty research grant of UNTHSC to S.Chu)

Sponsor:

406

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TISSUE REMODELING AND CONTRACTION IN VITRO STUDIES

Purpose: The main objective of these studies is to demonstrate that fibroblasts immortalized by stable expression of human telomerase reverse transcriptase (hTERT) participate in tissue remodeling in the same way as infant dermal fibroblasts by becoming activated to myofibroblast phenotype.G

Methods: The expression of cytoskeletal proteins in sparse and densely populated monolayers of infant dermal and hTERT fibroblasts were determined by indirect immunofluoresence and western blot analysis. Collagen synthesis by both cell types was measured using pic ric acid with sirius red assay(PSR assay) after stimulation with ascorbic acid and ascorbic acid phosphate. Tissue contraction was determined after exposure of connective tissue equivalents (CTE), to serum, LPA, and TGFb by measuring the loss in wet weight. Pre-treating the cells with mannose 6-phosphate inhibited tissue contraction..!

Results: Increased expression of a-smooth muscle actin was observed in low-density monolayer cultures. The maximal activation time to this wound healing phenotype in three-dimensional CTE was 6 hours. At this time point, increasing amount of serum increased the CTE contraction. LPA and TGFb are two major cytokines that contracted CTE. Incubation of cells with mannose-6-phosphate and de-lipidation of serum inhibited this CTE contraction.□

Conclusions: Low-density monolayer cultures model the cell invasion into the wound and show phenotype transition into myofibroblasts. The residential time in the 3-D environment regulates the ease of transition to myofibroblasts supporting the hypothesis that CTE is an appropriate in vitro model of wound healing. Major serum components that activate fibroblast during tissue repair are LPA and TGFb. LPA acts through small G protein coupled receptor and TGFb through the Smad 3 pathway. Effective inhibition of tissue contraction can be achieved once the signaling mechanisms involved are understood. hTERT fibroblasts are an excellent substitute for infant dermal fibroblasts for wound healing studies.*o

Sponsor: *Tobacco Grant & Global Medical Research*

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REPAIR IN THE NEURONAL TISSUES-ARE ASTROCYTES "FIBROBLASTS" OF NEURONAL TISSUE?

Purpose: The objectives of these studies are to demonstrate that a. specific differentiation of human neurosphere produces cells that express astrocyte markers; b. these astrocytes are proliferative and adopt a cytoskeleton that prepares them for tissue repair and c. the astrocytes can be incorporated and survive as components of a three dimensional model. h□

Methods: Commercially available human neonatal neurospheres are cultured in a medium containing EGF, progesterone insulin, transferrin and fetal bovine serum (FBS, 5%). The resulting astrocytes were subjected to indirect immunofluorescence and western blot analys es using antibodies for specific astrocyte markers, cytoskeletal proteins and PKC isoforms. Astrocytes were incorporated in to collagen type I three-dimensional matrix and shown to remain viable within the gels.t

Results: In the astrocyte growth medium (AGM) the neurospheres attach and the component cells slowly differentiated over a period of weeks into astrocytes. The astrocytes are proliferative and can be subcultured for expended periods of time (number of passages) b ut have to be seeded throughout at high cell density. Astrocytes were shown to stably express glial fabrillary acidic protein (GFAP), a-internexin, and b-tubuln. Additionally these cells also express cytoskeletal proteins vimentin (Vm), and myosin ligh t chain (MLC). A

Conclusions: Differentiation of stem and progenitor cell populating hollow human neurospheres into astrocytes is an attachment dependent process. The attachment and differentiation are probably due to the presence of progesterone and FBS. The proliferation and survi val in high-density cultures is consistent with activation of the cell cycle and migration to populate the wound bed from the cell rich surrounding tissue during the repair process. Assembly of a cytoskeleton similar to that of myofibroblasts suggests t h at astrocytes can be migratory and should be able to initiate tissue contraction, another characteristic of wound healing phenotype. The astrocytes also express their typical profile of neuronal markers such as GFAP. The putative differentiation sign a li ng might involve PKC regulated pathway and the profile of these signaling molecules shows expression of the following PKC isoforms: a and bI strongly, bII and h, weakly and z and e very weakly.dv

Sponsor: *Tobacco Grant & Global Medical Research*

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PROTEIN KINASE C D DOWNREGULATION IN PARENTAL AND CISPLATIN RESISTANT HELA CELLS.

Purpose: Protein kinase C (PKC) belongs to a family of serine/threonine kinases which are activated by extracellular signals. The PKC isoforms have been classified into three distinct subfamilies : Conventional PKCs (cPKC) comprising a , b1 and the splice variant b2 , and c ; novel PKCs (nPKC) delta d , e , h and q ; atypical PKCs (aPKC) z and l . The conventional PKCs require calcium ions and diacylglycerol (DAG) in addition to phosphatidylserine (PS) for activation, while the novel PKCs require diacylglycerol and phosphatidylserine for activation . The atypical PKCs require only phosphatidylserine for activation . PKCd is a novel PKC and plays an important role in mediating apoptotic cell death . PKCd can be activated by tumor promoting phorbol esters, such as 12-O-tetradecanoylphorbol -1-acetate (TPA) and phorbol 12,13-dibutyrate (PDBu) but prolonged cellular exposure to phorbol esters cause a downregulation of PKCs. Our labaratory has shown that prolonged cellular exposure to TPA or PDBu decreased PKCd level in cisplatin-sensitive Hela cells but not in cisplatin-resistant Hela/CP cells . I have examined if lack of decrease in PKCd in Hela/CP cells was due to an increase in synthesis or decrease in degradation of PKCd.

Methods: Hela and Hela/CP cells were cultured in nine well plates and pretreated with 20microgram/ml of cyclohexamide and 10micromolar of MG132. These cells were then treated with 1micromolar PDBu for one hour and five hours in different wells. The cells were processed and treated with lysis buffer after which the protein concentration was determined Bradford method. About 20 microgram of protein was loaded on to a gel and electrophoresis done. It was then transferrred on to a nitrocellulose paper and western blot done using PKC delta polyclonal antibody

Results: In Hela cells, PDBu induced downregulation of PKC delta which was prevented by the proteasome inhibitor MG132. The level of PKC delta in cells treated with MG132 and PDBu was less when cells were pretreated with cyclohexamide.

Conclusions: We are currently examining the effect of cyclohexamide and MG132 on PDBu induced downregulation of PKC delta in Hela/CP cells.

Sponsor:

409

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REGULATION OF PKCN

Purpose: Our lab had previously found that PKCn was stable to the degradation compared to other PKCs. We hypothesize the stability of PKCn is because of its phosphorylation.

Methods: We treated MCF7-Cells with activators of PKCs (PDBu, TPA, ILV, bryostatin I) or inhibitors of PKCs (BIM, Rottlerin, Gö6976) or inhibitors of other protein kinases (LY 294002, Rapamycin, PD98059, KT5720, AG1288) or protease inhibitors (MG132, Calpeptin, zVAD-fmk) with BIM or phosphatase inhibitors (Calyculin A, Okadaic acid) with BIM. After each treatment, we harvested and lysised the MCF7-cell. We performed western blot and analyzed the PKCn levels.

Results: PKC activators increased PKCn level. BIM, a PKC inhibitor, reduced PKCn level. However, inhibitors of cPKC, nPKCd, MAPK, PKA, Tyrosine kinase, mTOR did not cause the decrease of PKCh level. The treatment with phosphatase inhibitors blocked the effect of BIM, but the treatments with protease inhibitors or caspase inhibitors did not prevent the effect of BIM.

Conclusions: We conclude that PKCn is stabilized by phosphorylation and removal of phosphate residues by ser/thr phosphatase leads to its degradation.

Sponsor:

410

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NEURONAL COMMITMENT IN HUMAN NEUROSPHERE CELLS IS REGULATED BY FGF2 VIA A PKC MEDIATED PATHWAY

Purpose: The objective of these studies is to demonstrate that differentiation of the cells populating human neurospheres is regulated by protein kinase C (PKC). Dissecting the signaling cascades and identifying isoform (s) responsible for a specific direction of commitment will facilitate selection of the predominant neuronal progeny after attachment and differentiation.

Methods: Human neonatal neurosphere were incubated for 9 days in the presence of 1,5, and 20ng/ml FGF2, 40ng/ml EGF or grown as monolayers on FNC or collagen type IV plated surfaces. The PKC expression profile was determined by western blot analysis of lysates using antibodies against a, bI, bII, g, d, e, h, and q isoforms. The neurospheres were then exposed to: a. PMA (50ng/ml), PKC inhibitor GF109203X (500nM) and PMA with GF109203X (50ng/ml and 500nM respectively) and b. 5ng/ml FGF2 in the absence and presence of GF109203X (500nM). Concurrently a-internexin, neurofilament M (NF-M) and b-tubulin expression, was determined by indirect immunofluorescence, confocal microscopy and densitometry.e

Results: PKCa, bI, bII, d, e, h, and q, were detected. PKC a and bII; a and e; bI and II: bI and e; bII and e, a and bII were co-expressed uniformly across the neurosphere wall. FGF2 upregulated the expression of PKC a (5ng/ml), bI, and h (20ng/ml). PKC bII d, e, and q were downregulated. Novel PKCs were unaffected by EGF but bII was upregulated. In outgrowth on FNC and collagen IV all classical and novel isoforms were suppressed but e, was upregulated on collagen IV. PMA upregulated the expression of a-internexin and b-tubulin (the effect on NF-M was insignificant), but GF 109203X inhibited the increases a-internexin and b-tubulin expression. GF 109203X alone increased levels of a-internexin and NF-M but not b-tubulin. GF 109203X completely ablated FGF2 induced upregulation of a-internexin and NF-M but b-tubulin was elevated.

Conclusions: The PKC profile (expression of a bI, bII, d, e, h, q) remains relatively constant throughout treatments but the expression levels vary. Stimulation of PKCs (PMA) and FGF2 upregulation of a-internexin and NF-M is ablated by PKC inhibition with GF 109203X), but b-tubulin expression is elevated. FGF2 mediated neuronal commitment is partially regulated by PKC, but the relationship is complex. PKCa and bI play a role in a-internexin and NF-M expression, but PKCa, d, e and q may be involved b-tubulin expression. PKCz, implicated in NGF mediated differentiation of PC12 cells, was not detected.

Sponsor: *N/A*

500

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THE PATIENT-PHYSICIAN RELATIONSHIP IN TYPE 2 DIABETES: KEY ASPECTS OF DISEASE PERCEPTION

Purpose: This study investigated relationships between somatic complaints, attitude toward diabetes, diabetes knowledge, and health care trust in patients with Type 2 diabetes.

Methods: Participants included 115 type 2 diabetes patients presenting to a Medical University Family Medicine clinic. Participants completed self-report questionnaires designed to measure Somatic Complaints, Perception of Diabetes Severity, Diabetes Knowledge, and Distrust in Health Care System and Providers. Number of Comorbid Conditions and most recent HbA1c were obtained from medical charts. Data was subjected to a Pearson Product Moment Correlation.

Results: Relationships between HbA1c or Number of Comorbid Conditions with Somatic Complaints, Perception of Diabetes Severity, Distrust of Health Care System or Providers, and Diabetes Knowledge were nonsignificant. Perception of Diabetes Severity was positively associated with Somatic Complaints ($r = .34$, $p < .00$) and with Distrust in Health Care System ($r = .19$, $p < .05$). Somatic Complaints was also positively associated with Distrust in Health Care System ($r = .26$, $p < .01$) but negatively associated with Diabetes Knowledge ($r = -.17$, $p < .05$) and Distrust in Health Care Providers ($r = -.19$, $p < .05$). Distrust in Health Care System was positively associated with Distrust in Health Care Providers ($r = .17$, $p < .05$) and Diabetes Knowledge ($r = .18$, p .05).

Conclusions: Perceptions of disease severity and experience of vague/generalized symptoms by diabetes patients appears independent of the degree to which metabolic control is achieved or the actual number of diabetes related complications. However, as patients become more excessively focused on somatic complaints, they perceive their diabetes to be of greater severity, but also tend to be less knowledgeable about the facts of diabetes and experience more generalized distrust of the health care system. While trust in the system does not appear to improve with increased knowledge about diabetes, it does improve with greater degrees of trust in the providers of health care services. In order to better manage diabetes patients with excessive somatic complaints, it appears important to focus on developing a trusting patient-physician relationship that could facilitate a more accurate understanding about the nature of the disease. The impact on health care utilization patterns of incorporating a more patient-centered approach into the routine management of these individuals should be further investigated.

Sponsor:

501

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NOVEL TARGETING MECHANISM OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE IIB

Purpose: To determine how different CaMKIIB isoforms are targeted to different cellular location.

Methods: Site-directed mutation, Adenovirus generation, Immunohistochemistry, Transfection, Protein purification, Microtubule binding assay

Results: Evidences from immunohistochemistry suggested that CaMKIIB and B' are targeted to actin filaments; while CaMKIIB'e is targeted to microtubules. However, CaMKIIBe predominantly found in cytosol. The targeting of CaMKIIB'e to microtubules required autophosphorylation but not its activity. This is further validated by microtubules binding assay. Purified CaMKIIB'e is found to co-precipitated with purified microtubules.

Conclusions: Differences in the variable regions of CaMKIIB determine cellular location of the proteins.

Sponsor: *American Diabetes Association*

502

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ACTIVATION OF CALCIUM/CLAMODULIN-DEPENDENT PROTEIN KINASE II VIA THE AMPLIFYING PATHWAY OF GLUCOSE-INDUCED INSULIN SECRETION

Purpose: Activation of CaMKII may contribute to the mechanism by which glucose drives insulin secretion through the KATP channel-independent "amplifying" pathway.

Methods: Routinely rat islets and pancreatic cell line INS-1 cells are used as model. Diazoxide is used to clamp KATP channels in an open or closed state. Okadaic acid is used as phosphatase inhibitor. Following methods were used to evaluate the involvement of CaMKII in glucose induced insulin secretionvia the KATP channel-independent pathway.

Islolation of islets

Perifusion of islets to study insulin secretion profile

Cell culture and permeabilization by alpha-Toxin

Insulin secretion and measurement of secreted insulin content by RIA (Linco Research) from perifusion experiments

CaMKII activation assay

Results: 1. Glucose activated CaMKII via the Amplifying pathway was shown in insulin secretion data from perifusion experiments

2. Glucose activated CaMKII in the presence of Tolbutamide.

3. A combination of leucine and glutamine activated CaMKII and insulin secretion in the presence of diazoxide/KCl.

4. ATP:ADP ratio also showed influence on CaMKII activation by calcium in permeabilized beta cells

5.Activation of CaMKII by glucose via the amplifying pathway was mimicked by okadaic acid.

Conclusions: This study suggests that the activation of CaMKII via glucose via KATP channel-independent pathway represents an important mechanism in the regulation of insulin secretion. Moreover, evidences is provided that changes in the phosphorylation state of CaMKII is mediated via inhibition of a protein phosphatase in response to increases in the ATP/ADP ratio generated by increased flux through the mitochondria. These data suggest that the coordinated action of glucose via both triggering and amplifying pathways support a key regulatory role of CaMKII in nutrient-induced insulin secretion.

Sponsor:

503

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EPIDEMIOLOGIC STUDY OF SEVERE TYPE II DIABETES IN LATINOS WITH EMPHASIS ON SOCIAL, BEHAVIORAL AND LIFE STYLE FACTORS

Purpose: Explore the effects of social and economic factors such as family cohesiveness, acculturation and family income on the severity of diabetes among a sample of Hispanic patients treated at two community health centers in Fort Worth, Texas.

Methods: Latino patients under treatment at two healthcare clinics in Fort Worth, Texas, whom have been treated for type II diabetes (non-insulin dependent), between the ages of 30 and 70, were asked by their physicians to voluntarily participate in this study. A consecutive sample was drawn from the moment the study fieldwork was launched and continued until the total sample size of 276 was reached. A total of 193 severe cases and 83 non-severe diabetics were included in the study.The findings from the responses of cases and controls, to the questionnaire designed for this study regarding acculturation and family cohesiveness will be assessed to determine a possible link between cultural and social factors and severity of diabetes among study subjects.

Results: The findings of this study indicate that the patients who smoke or receive food stamps are more likely to have severe diabetes (HGA1c >7).

Conclusions: The findings of this study are in concordance with the reviewed literature. The prevalence of diabetes in the Latino population is due to genetic, social and cultural factors. Further research is needed to include education, as a factor influencing health.

Sponsor:

504

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DEPRESSION AND COPING STYLE IN GERIATRIC TYPE 2 DIABETES PATIENTS

Purpose: The purpose of the present study was to investigate coping mechanisms and depressive symptomology in geriatric type 2 diabetes patients.
Methods: This study consisted of 35 participants (13 male and 22 female) ranging in age from 60 to 89 years old presenting to a medical university based family medicine clinic. The depression subscale from the Multidimensional Health Profile (MHP) was administered to assess degree of depressive symptomology and social support. The Coping with Health Injuries and Problems (CHIP) was administered to assess the degree of palliative coping.
Results: Results indicated that patients with increased symptoms of depression utilize palliative coping mechanisms to a greater degree than social support $F(1, 29) = 4.413$, $p = .044$.
Conclusions: It appears that geriatric patients who are experiencing depressive symptoms tend to rely more heavily on □self-help□ responses to alleviate the unpleasantness of the situation.
Sponsor:

600

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A CAREER AWARENESS PROGRAM TARGETING AFRICAN AMERICAN HIGH SCHOOL AND COLLEGE STUDENTS

Purpose: The purpose of this research is to propose a career awareness program to promote the physician assistant(PA)profession among African American high school and college students. The program has several purposes: 1) to inform African American students about the PA profession and to provide them with a viable career alternative; 2)to present the kind of preparative course work needed that would better prepare these students for entry into a PA program; and 3) to provide practicing African American physician assistants as role models for the students.

Methods: The target audience, African American students, is identified by guidance counselors on high school and college campuses. The career awareness program is presented as a series of modules over six days during 50 minute class periods. At the end of each lecture period, students are given a homework assignment, constituting learner participation and formative evaluation of each day of instruction. An assessment at the end of the program measures whether its learning objectives have been accomplished. The program concludes with a panel discussion between minority PAs and participating students. A summative evaluation in the form of an exit survey determines whether students found the course relevant to their needs and whether their attitudes and beliefs changed.

Results: There are 8 modules in this curriculum. Module 1 introduces students to the founders of the profession and the cultural elements that influenced the profession's conception. Module 2 presents professional aspects such as the physician/PA team model and scope of practice. Module 3 discusses the preparative courses needed to gain entry into a PA program. Students are introduced to a typical PA studies curriculum in module 4. Module 5 presents the economic benefits afforded the physician/PA team. Module 6 introduces the credentialing process, while module 7 gives students access to additional information about the profession. Finally, module 8 allows students to interact with minority PA role models.

Conclusions: It is anticipated that this career awareness program will positively impact the knowledge, attitudes and beliefs of the participating student as they consider the PA profession as a career option. To determine whether the program actually resulted in an increase in African American enrollment in PA programs would require a long-range research project comparing groups of African American students who did and did not participate in the program.

Sponsor:

603

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WEARING A FOOTBALL HELMET EXACERBATES THERMAL LOAD DURING EXERCISE IN HYPERTHERMIC CONDITIONS

Purpose: Exercise produces a metabolically mediated thermal load that is a function of workload and environmental conditions. Much of this heat is dissipated from the head; thus, we hypothesized that wearing a football helmet impairs thermal regulation during exercise in a hyperthermic environment resulting in increased core temperature (Tc), skin temperature on the head (Th), and heart rate (Hr).

Methods: Six subjects (age range=21-30 years) performed 30 min of exercise which included a 5 min warm-up, 15 min of intermittent sprinting at 120 % of their VO2max workload (15 sec on, 45 sec rest), and 10 min of active cool-down (walking). Four skin thermistors were placed at selected positions on the head to measure Th, and an esophageal probe was inserted to measure Tc. Heart rate was recorded continuously and blood pressures was recorded manually at predetermined times. Each subject performed the protocol on consecutive days: one day with a standard collegiate helmet and the other without the helmet in a climate controlled chamber at 39-40 °C. The order was randomized.

Results: Tc increased significantly from baseline to the end of sprinting exercise without the helmet ("1.16°C + 0.48°C) and remained elevated at the end of recovery ("0.70°C + 0.40°C). The helmet lead to a greater ($p<0.01$) increase both at the end of exercise ("1.76°C + 0.69°C) and at the end of recovery ("1.3°C + 0.47°C). Similarly, Th was increased more with the helmet than without the helmet at the end of sprinting ("1.5°C + 0.37°C vs. "0.88°C + 0.37°C, $p<0.01$) and at the end of recovery ("1.66°C + 0.58°C vs. "1.34°C + 0.37°C, $p<0.01$). Cardiovascular strain (HR) increased by about 20 bpm at the end of exercise while wearing the helmet.

Conclusions: These data demonstrate that wearing a football helmet in hyperthermic conditions rapidly augments the thermal load and cardiovascular strain produced by 15 min of intensive exercise leading to significant increases in Tc, Th, and Hr.

Sponsor:

604

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PREDICTING FINISHING TIMES FOR MARATHON RUNNERS

Purpose: To determine our ability to predict marathon finishing times based on various criteria of age, gender, ethnicity, lifestyle activity level, maintenance training, and total number of marathons run.

Methods: An 18-question survey was distributed to adults participating in the Fort Worth, Cowtown Marathon on February 22, 2003. Questions pertaining to age, gender, ethnicity (Caucasian, Other), lifestyle activity level (sedentary, moderate, heavy), maintenance training (d 10, 11-20, 21-40, e 41 miles / week), and total number of completed marathons (0, 1-5, 6-10, 11-20, e 21) were analyzed. Analyses included descriptive statistics and multiple linear regression using the backward step procedure so that the final model included only significant predictors. Independent variables were included in the final model if they were significant at the alpha = 0.05 level.

Results: Two hundred and fifty-seven of the 334 (77%) marathon runners who completed surveys, finished the race with an average time of 271.7 minutes (sd = 47.0). The mean age of runners completing the marathon was 42.2 years (sd = 10.6), and 231 (77.5%) were male. Significant predictors included in the final model were age ($p < 0.0001$), gender ($p < 0.006$), maintenance miles/week ($p < 0.0001$), and total number of marathons participated in ($p = 0.002$). Lifestyle activity level and ethnicity were not statistically significant predictors.

Conclusions: Using the final model, 21.4% of the runners' finishing times can be predicted by age, gender, maintenance miles / week, and total number of marathons run. For every year increase in age, the finishing time increased by 1.5 minutes. Men completed in less time than women, and as the number of maintenance miles per week and the total number of marathons increased, the finishing time decreased. Further research is needed to determine additional factors contributing to the finishing time for marathon runners. Competitive marathon runners and coaches should be educated on the proper training techniques and extraneous factors contributing to a runner's finishing time. Development of such educational programs could improve times and refine training regimens.

Sponsor:

605

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PERCEPTION VS. REALITY: EFFECTIVENESS OF DIRECTED INFORMATICS TRAINING AS MEASURED BY STUDENTS□ PERCEIVED VS. ACTUAL SKILLS IN SEARCHING MEDLINE

Purpose: This study was conducted to determine the effectiveness of a problem-based approach to teaching third-year medical students to use MEDLINE. Objectives were to increase the effectiveness of students□ MEDLINE searches and to compare students□ perceived levels of search competence with their actual performance.

Methods: Students completed a patient interaction scenario based on a problem-based learning model, which required students to locate literature pertinent to the case. No instruction was given to the students before they began the exercise. Students emailed their search history and selected citations to librarian facilitators in sequenced segments. After the case segments, students attended a review session where they received feedback and search demonstrations. Students completed online pre- and post-surveys and conducted the same sample search, recorded by screen-capturing software, at the beginning and end of the course. Seven procedures embedded in the survey enabled comparison of the learners□ perceived and observed knowledge and performance prior to and following the intervention.

Results: Seven skills from the survey were matched to the observed performance in the recorded searches, scored as either positive or negative. T-tests on the pre- and post-surveys showed that students believed that they held a statistically significant amount of MEDLINE searching knowledge before and after the intervention. However, a t-test of perceived (P) vs. observed (O) measures revealed that students□ perceptions of their searching skills were inflated by several orders of magnitude above their actual performance abilities (pre-test P/O mean=9.664-8). Further, the gap between perceived and observed skill levels was more than twice as great after the intervention than before (post-test P/O Mean=1.079-19).

Conclusions: Students' ability to judge their own skills at searching MEDLINE is limited. Although students believe that they are aware of and can employ the elements that comprise good search techniques, they actually do not use the concepts and tools required to return comprehensive and precise results in MEDLINE. However, the problem-based learning approach effectively raises both perceived and actual searching skill levels. Comments from students showed a highly positive response to this type of teaching intervention. Librarians should continue to instruct students in MEDLINE search skills and to refine their teaching methods to help close the gap between perceived and actual skill levels.

Sponsor:

700

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INVOLVEMENT OF P38 MAP KINASE IN ET-1 MEDIATED APOPTOSIS OF RETINAL GANGLION CELLS

Purpose: Previous studies from our laboratory have shown ET-1 treatment promotes apoptosis of cultured rat retinal ganglion cells. The purpose of this study was to determine if p38 MAP Kinase is involved in ET-1 mediated apoptosis of retinal ganglion cells.

Methods: Virally transformed rat retinal ganglion cells (RGC5) were treated with 100nM ET-1 for 5, 10 and 15 min.Activation of p38 MAP Kinase was determined by immunoblotting using anti phospho p38 antibody. p38 MAP Kinase assay was carried out using ATF-2 as substrate in the presence of ^{32}P. In addition, the kinase assay samples were separated by SDS /PAGE (10%) and the radioactivity incorporated into ATF2 was detected by autoradiography.

Results: An increase in p38 phosphorylation was observed following ET-1 (100nM) treatment for 5 min in RGC5 cells, which was restored to control levels by 10 min. A corresponding increase in ATF-2 phosphorylation at 5 min was observed.The autradiography results of ATF-2 showed a similar increase in phosphorylation of ATF2 after ET-1 treatment for 5 min.

Conclusions: Activation of p38 MAP Kinase in RGC5 cells following the treatment with ET-1 (100nM) may contribute to ET-1 mediated apoptosis in RGC5 cells.

Sponsor:

701

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INVOLVEMENT OF MAPK AND PKC IN ENDOTHELIN-INDUCED ASTROCYTE PROLIFERATION

Purpose: Endothelins (ETs) have ionotropic, chemotactic and mitogenic properties. In particular, ET-1 is a potent mitogen for many cells especially when ET-1 levels are elevated under pathophysiological conditions. However, the signal transduction pathway utilized by ET-1 in astrocyte proliferation is not clear. In the present study, the signaling pathways involved in ET-1-mediated astrocyte proliferation were determined.

Methods: Specifically, we focused on the involvement of the MAPK, PKC and PI3 kinase signal pathways in cell proliferation after treatment with ET-1 in both U373MG astrocytoma and human optic nerve head astrocytes (hONAs) in culture. A formazan MTT assay was used for quantifying cell proliferation. Phosphorylation of ERK1/2, PKC, and Akt was detected by western blot analysis. A kinase assay was employed to detect the activities and translocation of PKC in membrane and cytosolic fractions. Changes in [Ca2+]i levels were determined by Fura-2 calcium imaging.

Results: ET-1 stimulated cell proliferation both in U373MG and in hONA cells in a MAPK and PKC-dependent manner. ET-1 caused a rapid phosphorylation of ERK1/2, which could be blocked by treatment with PD98059 and U0126 (a MEK inhibitor), in both cell types. While PKC inhibitor chelerythrine attenuated ET-1-induced cell proliferation, it was unable to block ET-1-induced ERK phosphorylation. In U373MG cells, ET-1 did not activate PKCs (c- and n-PKCs) and did not elevate [Ca2+]i. U73122 (a phospholipase C inhibitor) also had no effect on ET-1-induced ERK1/2 phosphorylation. FTI-277, a Ras inhibitor, and genistein, a protein tyrosine kinase inhibitor, did not abolish the ERK1/2 phosphorylation. LY294002, a PI3K inhibitor, completely blocked the phosphorylation of Akt and cell proliferation, but did not block the phosphorylation of ERK1/2.

Conclusions: ET-1 activates phosphorylation of ERK1/2, which plays an important role in astroglial proliferation for hONAs and U373MG astrocytoma cells. Conventional and novel PKCs appear not to be involved in astrocyte cell proliferation in U373MG cells. The PI3 kinase pathway is involved in signal transduction induced by ET-1, but it does not appear to participate in crosstalk with the MAPK pathway. The mitogenic effects of ET-1 may provide insight into ET□s role in astrogliosis leading to optic nerve damage as seen in glaucoma.

Sponsor: *EY11979 (TY); UNTHSC Intramural Grant (GP); AHAF G200006P (GP)*

702

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CHARACTERIZATION OF RAT RETINAL GANGLION CELLS WITH A DIFFERENTIATED MORPHOLOGY

Purpose: Previously we have shown that transformed rat retinal ganglion cells (RGC-5) undergo a morphological change after co-culture with human non-pigmented cilliary epithelial (HNPE) cells. The purpose of this study was to characterize these morphologically different RGC-5 cells using functional assays and immunocytochemistry.

Methods: HNPE cells were seeded on collagen inserts in DMEM complete medium and added to RGC-5 cells seeded on glass coverslips in 6-well plates. After 3 days of co-culture the wells were observed by light microscopy and pictures were taken. Changes in intracellular calcium concentrations were measured in response to 50¼M glutamate using Fura-2 calcium imaging in the presence and absence of MK-801, a NMDA receptor antagonist. Whole cell patch clamp technique was employed to detect changes in current following glutamate treatment. Immunocytochemistry was performed using antibodies against Thy-1.

Results: There was an increase in intracellular calcium concentrations in response to 50¼M glutamate. Pretreatment with MK-801 inhibited the glutamate-induced increase in intracellular calcium. Treatment of glutamate caused an inward current across the membrane in RGC-5 co-cultured with HNPE. The morphologically different RGC-5 cells expressed Thy-1.

Conclusions: RGC-5 cells upon co-culture with HNPE develop a differentiated phenotype which is responsive to glutamate. The NMDA receptor may be involved due to the inhibition of the calcium response with the treatment of MK-801. Although these cells change morphology they continue to express Thy-1 which is a characteristic retinal ganglion cell marker. Further characterization of these cells and the channels involved are currently being conducted using whole cell patch clamping, western blotting and immunocytochemistry.

Sponsor: *NEI EY11979*

703

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GENE EXPRESSION IN HUMAN TRABECULAR MESHWORK CELLS, LAMINA CRIBROSA CELLS AND OPTIC NERVE HEAD ASTROCYTES FOLLOWING EXOGENOUS BMP-4 TREATMENT

Purpose: BMPs , BMP receptors, and BMP associated proteins are expressed in the human TM and ONH, and bmp4+/- mice have been reported to develop elevated IOP and ON abnormalities.The purpose of this study is to characterize and compare changes in gene expression of human trabecular meshwork cells(HTM),lamina cribrosa cells(LC) and optic nerve head astrocytes (ONHA) in response to exogenous BMP4.

Methods: HumanTM cells, LC cells, and ONHAs were exposed to either vehicle or BMP -4(20ng/ml) for 24 hour and 72 hours, and total RNA was extracted using Trizol. Affymetrix Human Genome U133 GeneChip and UIMaDS were used to evaluate and compare changes in gene expression.

Results: A total of 39,000 genes and expressed sequence tags(ESTs) were examined. The data were filtered according to statistically significant changes in gene expression as well as a 2-fold or greater change in gene expression.In TM cells after 24 hours exposure to BMP4, a total of 105 genes were up regulated and 42 genes were down regulated. In LC cells after 24 hours of exposure to BMP4,a total of 278 genes were up regulated and 118 genes were down regulated.In ONHA after 72 hours of exposure to BMP4, 48 genes were up regulated and 193 genes were down regulated. Categories of genes with BMP4, induced altered gene expression included :(A) extracellular matrix /cytoskeleton,(B)growth factors and growth factor receptors and (C) signaling pathways.

Conclusions: The gene microarray data revealed BMP-4 induced changes in gene expression for a number of genes,including extracellular matrix/cytoskeletal components and growth factor related genes. The simultaneous screening of thousands of genes by the Microarray analysis would help identify gene(s) primarily involved in BMP signaling and will enhance our understanding of the role of bone morphogenetic proteins in the normal regulation of tissues involved in the pathogenesis of glaucoma.

Sponsor: *NIH Grant EY 12783 ,Alcon Research Ltd., Fort Worth, Tx*

704

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ENDOTHELIN-1 UPREGULATION OF APOPTOTIC TRANSCRIPTION FACTORS IN RETINAL GANGLION CELLS

Purpose: Endothelin-1 (ET-1) treatment has been shown to induce apoptosis in retinal ganglion cells. The purpose of this study was to determine the expression profiles of transcription factors upon treatment with ET-1, with a view to understand their possible role in apoptosis of retinal ganglion cells (RGC).

Methods: Retinal ganglion cells (RGC-5 cells) were treated with 100nM ET-1 for 24 hours. Transcription reporter arrays were used to analyze the simultaneous activation of multiple transcription factors. To confirm these findings, RGC-5 cells were treated with 1nM, 10nM, and 100nM ET-1 for 24 hours. Following treatments, electrophoretic mobility shifts assays were performed on nuclear extracts from the treated cells, for activator protein-1 (AP-1) and Smad 3/4 transcription factors.

Results: An upregulation of several transcription factors was observed in the transciption reporter array, after RGC-5 cells were treated 24 hours with ET-1. The most prominent increase was observed in AP-1, Smad 3/4 and MEF-1 transcription factors. ET-1 treatment (10nM and 100nM) of RGC-5 cells for 24 hours showed an appreciable increase in AP-1 binding activity, compared to untreated controls.

Conclusions: Administration of ET-1 to RGC-5 cells stimulates the expression of numerous transcription factors, particularly AP-1 and Smad 3/4, which may play an important role in apoptosis of retinal ganglion cells.

Sponsor: *NEI-EY11979*

705

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MRNA EXPRESSION OF EXTRACELLULAR MATRIX COMPONENTS BY NORMAL AND GLAUCOMATOUS HUMAN OPTIC NERVE ASTROCYTES AND LAMINA CRIBROSA CELLS

Purpose: Primary open angle glaucoma (POAG), a common form of optic neuropathy, is characterized by irreversible and progressive loss of axons from retinal ganglion cells (RGCs), usually in response to abnormally elevated intraocular pressure (IOP). There is substantial evidence that damage to optic nerve axons occurs at the level of the lamina cribrosa in the optic nerve head. In the glaucomatous optic nerve, cupping of the optic disc and compression, stretching, and rearrangement of the cribriform plates of the lamina cribrosa occur in response to elevated IOP. Remodeling of the optic nerve head in glaucoma involves astrocyte responses and changes in the extracellular matrix (ECM) composition and distribution. Changes in extracellular matrix may play an important role in the progression of the glaucomatous process and may be a causative agent in the pathogenesis of the disease. The purpose of this study was to demonstrate mRNA expression of extracellular matrix components by normal and glaucomatous optic nerve astrocytes and lamina cribrosa cells.

Methods: Synthesis of cDNA and reverse transcription polymerase chain reaction (RT-PCR) were conducted using total RNA obtained from well-characterized human optic nerve astrocytes (ONA) and LC cell lines from normal and glaucomatous human donors.

Results: mRNA for collagen IV, elastin, fibronectin and laminin were detected in normal and glaucomatous ONA and LC cell lines.

Conclusions: Normal as well as glaucomatous ONH astrocytes and LC cells expressed mRNA for extracellular matrix components. Further studies including quantitative PCR need to be done to demonstrate any alteration of extracellular matrix components in glaucoma and their probable role in the pathogenesis of glaucoma.

Sponsor: *NIH Grant EY12783 and Alcon Research Ltd., Fort Worth, TX*

706

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IN VITRO EFFECT OF FIBROBLAST GROWTH FACTOR-9 (FGF-9), CILIARY NEUROTROPHIC FACTOR (CNTF), AND INTERLEUKIN-1 ALPHA (IL-1 ALPHA) ON HUMAN OPTIC NERVE HEAD ASTROCYTES

Purpose: Glaucoma is a leading cause of blindness worldwide. A major risk factor for glaucoma is increased intraocular pressure that leads to pathological changes in the optic nerve head (ONH). Astrocytes within the ONH become activated in glaucoma and may cause detrimental changes to retinal ganglion cell axons. The factors that may cause activation of the ONH are unknown. The purpose of the research was to determine if exogenous FGF-9, CNTF, and/or IL-1 alpha activate human ONH astroctyes.

Methods: Three human ONH astrocyte cell lines were grown until approximately 80% confluent and treated for 48 hours with either CNTF (150ng/mL), FGF- 9 (2ng/mL), or IL- 1 alpha (2ng/mL) in serum-less media. Untreated cell lines acted as controls. RT-PCR was used to determine mRNA expression of CNTF, FGF- 9, and IL-1 alpha, and their respective receptor complexes. Western Blot analysis was used to demonstrate the presence of CNTF, FGF-9, and IL-1 alpha, their respective receptor complexes, and GFAP. Phase contrast microscopy was used to examine cell morphology. Proliferation assays was performed to demonstrate cell proliferation in response to exogenous CNTF, FGF-9, and IL-1 alpha.

Results: mRNA for CNTF, FGF-9, IL-1 alpha and their receptor complexes is expressed by human ONH astrocytes. Protein expression for CNTF and its receptor gp130 and LIFR and IL-1 alpha and its receptor IL-1RI was obtained via western blots. Protein expression for FGF-9, FGFR1 and FGFR4 was observed but FGFR2 and FGFR3 was lacking. Exogenous IL-1 alpha caused a significant increase in ONH astrocyte cell proliferation. Exogenous IL-1 alpha caused human ONH astrocytes to extend more processes.

Conclusions: These studies demonstrate that mRNA and protein for CNTF, FGF-9, and IL-1 alpha and their respective receptors are expressed by human ONH astrocytes. In addition exogenous IL-1 alpha caused morphological changes and increased proliferation. This research may help us understand the pathophysiology of the optic nerve head in glaucoma.

Sponsor: *National Institute of Health Grant #EY12783/ Alcon Research Ltd; Fort Worth, Texas*

707

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ROLE OF NON-FEMINIZING ESTROGEN ANALOGUES IN NEUROPROTECTION OF RAT RETINAL GANGLION CELLS AGAINST

Purpose: To compare and characterize the neuroprotective effects of 17beta-estradiol and novel estrogen analogues against glutamate induced cytotoxicity of rat retinal ganglion cells (RGC-5 cells).

Methods: RT-PCR, PCR-Southern Blot, and immunocytochemistry were used to determine the expression of estrogen receptors a and b in RGC-5 cells. RGC-5 cells, 17beta-estradiol, ICI estrogen receptor antagonist, three novel estrogen analogues (ZYC-1, ZYC-3, and ZYC-10), and L-glutamic acid were used for these studies. RGC-5 cells were pretreated with 17beta-estradiol or one of the estrogen analogues followed by an insult with L-glutamic acid (5 mM). Cell viability was assessed using the neutral red dye uptake assay. ICI compound was used, as an antagonist of estrogen receptors, to assess their involvement in neuroprotection. Levels of gamma-glutamylcysteinsynthetase in cells pretreated with estrogen analogue ZYC-3, followed by glutamate challenge, were monitored by immunoblot analysis.

Results: RGC-5 cells were shown to express both a and b estrogen receptors. Glutamate treatment resulted in 50% RGC-5 cell death. 17beta-estradiol and the three estrogen analogues protected the RGC-5 cells against glutamate cytotoxicity in a dose dependent manner that was minimally inhibited by the estrogen receptor antagonist ICI. The efficacy of neuroprotection by the estrogen analogues was as follows: ZYC-3 >ZYC-10 > ZYC-1. In RGC-5 cells preincubated with ZYC-3 followed by a glutamate challenge, the protein levels of the catalytic and regulatory subunits of gamma-glutamylcysteinsynthetase were increased.

Conclusions: 17beta-estradiol and non-feminizing estrogen analogues ZYC-3, ZYC-10, and ZYC-1 protect RGC-5 cells against glutamate cytotoxicity. These compounds appear to affect their neuroprotection via an antioxidant pathway. The data support the hypothesis that estrogen analogues may be useful in the neuroprotection of retinal ganglion cells in ocular pathologies such as glaucoma.

Sponsor: *National Institutes of Health - National Institute of Aging*

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GLUCOCORTICOID RESPONSIVENESS IN GLAUCOMA IS REGULATED BY THE NUCLEAR EXPRESSION OF GLUCOCORTICOID RECEPTOR BETA IN TRABECULAR MESHWORK CELLS

Purpose: The ocular administration of a glucocorticoid (GC) results in an increase in intraocular pressure in greater than 90% of Primary Open Angle Glaucoma (POAG) patients as compared to 30-40% of the general population. Why these patients respond to this high rate is not clear. GC responses are mediated through GC receptor alpha (GR alpha). However, GC receptor beta (GR beta) is associated with GC resistance in many diseases,including asthma. Currently we are investigating the potential role of GR beta in regulating GC responsiveness in glaucoma.

Methods: 5 Normal and 6 glaucomatous TM cell lines were used.Western blots were performed to investigate the cytoplasmic and nuclear expression of GR alpha and GR beta and their regulation by dexamethasone (Dex), a synthetic glucocorticoid. Immunofluoreacence microscopy (IFM) was used to compare the expression of GR beta between normal and glaucomatous TM cell lines. Up-regulation of GR beta expression was conducted by transfection of GR beta expression vector in glaucomatous TM cell lines. The effect of GR beta on Dex-induced expression of reporter gene was investigated using luciferase assay, and the effect on Dex-induced expression of a glaucoma gene, myocilin, was evaluated using confocal IFM and Western Blot.

Results: Western blot detected that GR beta was expressed in all these trabecular meshwork cell lines. GR beta was expressed not only in the nucleus but also in the cytoplasm. Interestingly, most normal TM cell lines had relatively high amount of GR beta expression, especially in the nucleus compared to glaucomatous TM cell lines. Dex treatment increased the expression of GR beta in normal TM cell lines but not in glaucomaous TM cell lines, while caused the translocation of GR alpha from the cytoplasm to the nucleus and also a time-dependent down-regulation of GR alpha. Strikingly, overexpression of GR beta in glaucomatous TM cell lines inhibited the Dex-inducted expression of reporter gene luciferase and a glaucoma gene, myocilin, in glaucomatous TM cells.

Conclusions: We have, for the first time, detected the close association between the low expression of GR beta and hyper-responsiveness to GCs in POAG subjects. The up-regulation of the GR beta expression in glaucoma TM cells can inhibit cellular responses to GCs. These findings demonstrate that decreased nuclear amounts of GR beta could result in the enhanced regulatory activity of GC/GR alpha and contribute to GC hyper-responsiveness seen in glaucoma patients.

Sponsor: *NEI EY11979*

709

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RETINAL PROGENITOR CELLS EXPRESS HIGH LEVELS OF P75NTR IN VITRO AND IN DEVELOPING RETINAS

Purpose: Retinal neurons and Müller glia are derived from a common population of retinal progenitor cells (RPCs) that possess a multilineage differentiation potential and contribute to the production of different retinal cell types in a defined histogenetic order. While retinal development is controlled by the interplay of mechanisms that regulate proliferation, differentiation, or cell death of RPCs, the exact molecular mechanisms remain to be elucidated. Recent studies have shown that the low affinity neurotrophin receptor, p75NTR, might be involved in apoptotic cell death in the developing mammalian retina. Studies in our laboratory further showed that a novel intracellular signaling pathway involving p75NTR, NRAGE (a p75NTR-adaptor protein), p53 (a tumor suppressor), and Bcl2 proteins mediate photoreceptor cell death in retinal disease. In the following study, we have begun to investigate the role of the novel p75NTR intracellular signaling pathway during programmed cell death in the developing retina.

Methods: RPCs isolated from postnatal day 2 rat retinal explants were characterized for expression of photoreceptor markers and p75NTR-associated molecules including NRAGE, p53, Bax and Bcl2, using immunohistochemistry and immunoblotting. The protein expression of the above molecules were also investigated in vivo in developing rat retinas.

Results: Rat RPCs expressed low levels of rhodopsin and high levels of Pax6 consistent with their undifferentiated state. RPCs also expressed high levels of p75NTR, NRAGE, p53 and Bcl2. The inverse relationship between the temporal expressions of rhodopsin and p75NTR was verified in the developing rat retinas showing high levels of p75NTR in the neuroblastic layer of the outer retina containing rhodopsin negative-RPCs and apoptotic cells. Later, with further differentiation of the neuroblastic layer, increased expression of rhodopsin with decreased p75NTR was observed in the neuroblastic layer.

Conclusions: Our study showed that during development, high levels of p75NTR and p75NTR-associated molecules were observed in undifferentiated RPCs in culture and in immature rat retinas. Our study supports the hypothesis that p75NTR signaling pathways may play an important role in retinal development by modulating programmed cell death and/or differentiation.

Sponsor: *Not aplicable*

710

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EFFECTS OF GROWTH FACTORS ON RAT RETINAL PROGENITOR CELLS

Purpose: Restoration of sight in patients suffering from age-related macular degeneration (ARMD) and retinitis pigmentosa (RP) by transplantation therapies has not been successful to date. Development of retinal cells for transplantation in diseased eyes remains a primary focus of this laboratory.

Methods: Progenitor cells were isolated from retinal explants of postnatal day 2 rats grown in proteins secreted by retinal pigment epithelial (RPE) cells. These progenitor cells were transformed using the psi 2E1A virus, passaged and frozen in liquid nitrogen. Progenitor cells from passage 5-15 were used for the in vitro and proliferation studies.

Results: Retinal progenitor cells grown in serum were shown to express high levels of PAX6, which is a transcription factor that maintains a cell ability to proliferate, without affecting differentiation. Some cells expressed the photoreceptor marker opsin, although at low levels. Proliferation bioassays showed that progenitor cells grown in serum and RPE-secreted proteins proliferated 4 and 7 fold, respectively. Progenitor cells grown in serum exhibited processes, however, cells cultured in the secreted proteins remained round and grew in small clusters. Cells from these clusters returned to a serum-containing media showed a typical process-bearing morphology and continued to proliferate. Cells cultured in stem cell factor (SCF) proliferated and exhibited a small, round cell body, with fine processes. In sharp contrast, cells grown in leukemia inhibitory factor (LIF) showed large processes and larger cell bodies. Brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (FGF-2) promoted the elaboration of fine processes from progenitor cells, but little-to-no cell proliferation.

Conclusions: These studies demonstrated that progenitor cells of neonatal rat retinal explants were capable of being virally transformed and survived storage in liquid nitrogen for over one year. In addition, transformed cells exhibited an immature cell character, PAX6, and proliferated extensively when cultured in serum, RPE secreted proteins, and stem cell factor. These cells will be investigated to determine their utility for transplantation in diseased rat retinas.

Sponsor:

711

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SECRETION OF PRO-NERVE GROWTH FACTOR AND EXPRESSION OF PROPROTEIN CONVERTASES BY CELLS OF THE OPTIC NERVE HEAD

Purpose: The mechanism for the death of retinal ganglion cells (RGC) in glaucoma remains to be elucidated. Recent data has shown that proneurotrophins are capable of enabling cell death via the p75 receptor pathway. We have shown previously that optic nerve head astrocytes (ONA) and lamina cribrosa (LC) cells express neurotrophins but lack expression of p75 (Lambert et al. IOVS, 42:2315-23. 2001). The purpose of this study is to examine the secretion of proneurotophins by optic nerve head cells as well as to characterize the expression patterns of the proprotein convertases (PC), an endoprotease family capable of converting proneurotrophins into their mature form.

Methods: The mechanism for the death of retinal ganglion cells (RGC) in glaucoma remains to be elucidated. Recent data has shown that proneurotrophins are capable of enabling cell death via the p75 receptor pathway. We have shown previously that optic nerve head astrocytes (ONA) and lamina cribrosa (LC) cells express neurotrophins but lack expression of p75 (Lambert et al. IOVS, 42:2315-23. 2001). The purpose of this study is to examine the secretion of proneurotophins by optic nerve head cells as well as to characterize the expression patterns of the proprotein convertases (PC), an endoprotease family capable of converting proneurotrophins into their mature form.

Results: The 32 kDa form of pro nerve growth factor is found in conditioned media from ONA and LC cells cultured for 48 hours. Using RT-PCR, mRNA for furin, PC1, PC5, and PC7 is expressed in both LC and ONA cell lines. PACE4 is expressed variably in both LC and ONA cell lines.

Conclusions: This study demonstrates for the first time that proneurotrophins are secreted from the LC and ONA cell lines. In addition, this study demonstrates that members of the PC family are expressed in these cell lines. This expression is unique from previous reported data on PC expression in glial cells. Therefore, it may be possible that proneurotrophin processing in the optic nerve head may be regulated differentially in glaucomatous conditions

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800

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PSYCHOLOGICAL CHARACTERISTICS OF PATIENTES SELECTING LAPAROSCOPIC BANDING

Purpose: The purpose is to describe the characteristics of Lap-Band surgical candidates utilizing the Millon Behavioral Medicine Diagnostic (MBMD), a measure developed for use in a variety of medical populations. The MBMD is a self-report inventory that contains 3 response pattern scales, and 29 clinical scales reflecting negative health habits,
psychiatric indications, and coping styles. This paper describes the Lap-Band patient in regards to the psychiatric indications scales.

Methods: Participants included 75 (13 men, 62 women) Lap-Band surgical candidates who presented for psychological evaluation to the Bariatric Surgical Clinic at UNTHSC. Each participant completed a standard assessment battery to which the MBMD was added.

Results: Prevalance scores for anxiety, depression, cognitive dysfunction, emotional lability and guardedness were compared to the standardized sample utilizing the nonparametric Kruskal-Wallis test. No significant differences between the groups were found.

Conclusions: Results from this study supported the hypothesis that Lap-Band surgical candidates are not signficantly different from the general medical population on 5 psychiatric indicators (anxiety, depression, cognitive dysfunction, emotional lability, and guardedness).

Sponsor:

801

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RUNNING RELATED INJURY OCCURRENCES AND MAINTENANCE TRAINING IN MARATHON RUNNERS

Purpose: To analyze the number of injuries in marathon runners and the time they completed the marathon by the number of miles run per week during maintenance training.

Methods: An 18-item survey was presented to Marathon runners of the February 2003 Cowtown Marathon in Fort Worth, TX to complete voluntarily. Variables of interest included age, gender, health problems, nutritional status, running experience, maintenance training, pre-race training habits and ever having had a running related injury. Groups were compared using chi-square statistics. The UNTHSC Institutional Review Board approved the survey methodology.

Results: The total number of surveys completed was 334. Ages ranged from 18 to 81 years with an average age of 42 years. Of the sample, 260 (77.8%) were male and 74 (22.2%) were female. Maintenance training was divided into four categories: d10 miles/week, 11-20 miles/week, 21-40 miles/week and e41 miles/week. Thirty-one runners (9.4%) ran d10 miles/week with 13 (41.9%) suffering an injury, 102 runners (31.0%) ran 11-20 miles/week with 64 (62.7%) suffering an injury, 167 runners (50.8%) ran 21-40 miles/week with 123 (73.7%) suffering an injury, 29 runners (8.8%) ran e41 miles/week with 24 (82.8%) suffering an injury. The overall chi-square value was 16.35 (pd0.001). Of the sample, 298 (89.2%) completed the marathon. The times for completing the marathon were divided into quartiles of 170 - 239.75, 239.76 - 268.5, 268.51 - 298.25, and 298.26 - 432 minutes. The average time for completing the marathon was 272.76 (SD = 47.17) minutes. There was a statistically significant difference in the number of runners completing the race in each quartile by maintenance training (miles / week), chi-square = 39.60 ($p<0.001$).

Conclusions: The data shows that the majority of runners ran 21-40 miles/week during maintenance training. The percent of runners in each group who suffered an injury indicates that more injuries occur when a runner runs more miles per week. Additionally, the percentage of runners completing the marathon in the first quartile of completers increases with the number of miles / week of maintenance training. Physicians and athletes need further education on correct maintenance training to maximize race performance and minimize the risk of injury.

Sponsor:

802

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PERSONALITY CORRELATES OF BODY MASS INDEX IN BARIATRIC PATIENTS

Purpose: Past research has suggested a link between pre-existing psychopathology (e.g depression and anxiety) and Binge Eating Disorder (BED); however, results have been equivocal. This study examined the relationship between elevations on scales 2 and 7 of the MMPI and body mass index (BMI)

Methods: 57 female bariatric patients were administered the MMPI following calculation of BMI during a pre-surgical screening. Individuals were divided into 2 groups (moderate-high vs. severe-high BMI) based on a median split on the BMI variable

Results: Results indicate that individuals in the severe-high BMI group scored significantly higher on MMPI scales 2 and 7 in comparison to people in the moderate-high BMI group

Conclusions: This finding is consistent with past literature which suggests that negative affect may contribute to obesity in some individuals.

Sponsor:

803

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IMPACT OF A RURAL EDUCATIONAL EXPERIENCE ON MEDICAL STUDENTS□ ATTITUDES TOWARD RURAL MEDICINE

Purpose: To determine the impact of participation in a rural educational experience, particularly the Rural Family Medicine Track or the rural-based family medicine clerkship, on medical students□ attitudes toward rural medicine.

Methods: A total of 31 students (21 Clerkship students and 10 Track students) from the medical school Classes of 1998, 1999, and 2000 completed surveys before and after completing their Year 3 family medicine clerkship at a rural training site. Six questions regarding changes in attitude were common to both questionnaires. All answers utilized a five-point Likert response scale ranging from □Strongly Agree□ (5 points) to □Strongly Disagree□ (1 point). Descriptive statistics and multivariate analysis were used to determine if changes in scores between the pre- and post-experience questionnaires were significant ($p < 0.05$). Responses were compared within groups (pre- vs. post-experience) and between groups (Track vs. Clerkship).

Results: Overall, there was a significant improvement in attitudes toward rural medicine and future plans to practice medicine in a rural community. The most statistically significant change in attitude was in the medical students□ plan to practice medicine in a rural community. This was significant both within the two groups (measuring pre- and post-experience) and between the two groups (Rural Track vs. Clerkship Only). Other significant attitudinal changes were an increased likelihood for practicing medicine in a rural community and that the experience in a rural community will benefit the student regardless of where he/she practices. These items were significant only within the two groups.

Conclusions: In both groups, attitudes about rural medicine and practicing in a rural community improved over time. This data suggests that participation in a rural medicine training experience, regardless of Clerkship or Track, has a beneficial impact. Continued monitoring of data as more classes participate in rural experiences will be critical to determining the effects of this type experience.

Sponsor:

804

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TREATMENT CREDIBILITY IN OSTEOPATHIC MANIPULATIVE TREATMENT RESEARCH

Purpose: Treatment credibility refers to the degree to which subjects believe that the treatments offered to them in competing arms of clinical trials are likely to be beneficial. We recently explored treatment credibility in a randomized controlled trial of osteopathic manipulative treatment (OMT) for chronic low back pain that included both sham manipulation and no-intervention controls to determine how confidence in treatment may affect trial completion and findings.

Methods: Written descriptions of osteopathic manipulative treatment (OMT) and sham manipulation were presented to subjects at trial baseline and exit. They were then asked to rate their confidence in each treatment using a weighted, five-point Likert scale: "strongly agree," (5); "agree," (4); "undecided," (3); "disagree," (2); and "strongly disagree," (1). The primary outcome measure was the credibility ratio (CR) and its 95% confidence interval (CI) for credibility in OMT relative to credibility in sham manipulation, as computed by the relative weights for responses to the two relevant items.

Results: Overall, the credibility ratio at baseline was 1.10 (1.03 - 1.16), indicating a small, but statistically significant, credibility differential favoring OMT over sham manipulation. Similarly, at trial exit, the credibility ratio was 1.15 (1.06 - 1.23). Changes in the credibility ratio over time were not significant (P = .36). There were no significant differences in CRs between trial completers and non-completers or among the three treatment groups. Further, there were no significant (treatment group) x (time) interactions.

Conclusions: Slightly elevated CRs suggested a small treatment credibility differential favoring OMT over sham manipulation; however, the results did not indicate that treatment credibility systematically affected trial results or attrition.

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900

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STROKE AND ITS RELATIONSHIP TO HYPERTENSIVE DISORDERS OF PREGNANCY

Purpose: The incidence of preeclampsia during pregnancy is 3-8%. One of its major complications is stroke. The purpose of this study was to describe the relationship between stroke and preeclampsia during pregnancy and postpartum; also, to discuss any long term consequences of having preeclampsia. Furthermore, a case presentation is reported. Finally, risk factors and treatment options are addressed.

Methods: A meta-analysis of three studies compared the risk of stroke in women who had hypertensive disorders of pregnancies and to those who did not. A second meta-analysis of two studies evaluated women who were preeclamptic/eclamptic during their first pregnancy and the long-term risk for stroke.

Results: a)1st meta-analysis: The first study was a population-based cohort study in Sweden between 1987 and 1995 which analyzed for the relative risks of stroke during pregnancy. It was found that during the third trimester, the RR of stroke was 12 times higher in preeclamptic patients when compared to patients who were nonpreeclamptic. The RR of stroke for patients with preeclampsia around the time of delivery was >600 times higher compared with nonpreeclamtic patients. Furthermore, when compared to nonpreeclamptic women, the RR of stroke was >6 times higher among women with preeclampsia during the puerperium. Other studies were examined which found similar findings.

b) 2nd meta-analysis: A cohort study of women who had preeclampsia during their first pregnancy from 1951 to 1970 was conducted in Scotland. Compared to the randomly sampled control group, the death from stroke for the preeclampsia/eclampsia group was 3.59 folds higher. Another cohort study was conducted from 1967 to 1992. The subjects were mothers recorded in the Norwegian medical birth registry and were divided into those who had preeclampsia and those who did not. The risk of death from stroke among women with preeclampsia and a preterm delivery was 5.08-fold higher (death from stroke and term delivery was 0.36-fold higher).

Conclusions: The risk during the pregnancy/postpartum period is 6 to 12 times higher, while the long-term risk is 2 to 5 times higher. Overall, there is a 3 to 12 times higher risk when compared to normal pregnancy. Since a link has been made between hypertensive disorders of pregnancy and stroke, further studies are needed on how to predict and treat, as well as prevent these occurrences from happening.

Sponsor:

901

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A SURGICAL APPROACH TO CEREBRAL VASCULAR ACCIDENT (CVA)

Purpose: Stroke is defined as a "brain attack" that happens when a part of the brain experiences a problem with blood flow. Disruption in blood flow deprives the brain tissue of oxygen and nutrients and within minutes brain cells begin to die. This is a medical emergency, with prompt treatment meaning the difference between life and death. Depending on the part of the brain affected, injury to the brain may include loss of speech, vision, or movement in an arm or a leg. I discuss a patient with atypical symptoms of bilateral carotid stenosis. The patient, a 55 year-old white male, presents with acute onset of vertigo and right-sided upper extremity weakness.

Methods: History of present illness, past medical history, and physical exam were assessed. Imaging of the person's brain was done using CT without contrast, MRI without contrast and carotid angiography. 95-99% stenosis was noted in the right internal carotid artery and 60-65% stenosis was noted in the proximal left internal carotid artery.

Results: Since the patient had bilateral stenosis of the internal carotid arteries, the question was which side to do the carotid endarterectomy(CEA) first. Even though the patient was having right-sided symptoms due to the left-sided stenosis, the surgeon decided to perform a right CEA first. Generally, the symptomatic side is treated (in this case the left stenosis); however, since there is a 95-99% occlusion of the right internal carotid artery, surgery was performed there first. If the left side had initially been performed, there was an immense possibility that the right side would become 100% stenosed leaving the brain infracted and untreatable. CEA is contraindicated for 100% stenoisis. Therefore, the right-sided surgery was performed first, with the left side having surgery three months later.

Conclusions: Immediate medical treatment for cerebrovascular accident (CVA) is essential. Thrombolytic therapy for an ischemic stroke must be administered within 3 hours of the event to not only improve the chance for survival, but also reduce the amount of disability resulting from the stroke. In addition to prescribing medication, surgery may be indicated for vessels that are moderately to severely narrowed by plaques. Based on the NASCET Study, (North American Symptomatic CEA Trial) in all individuals with carotid stenosis >70% or in individuals with 50-69% carotid stenosis and good candidates for surgery, a CEA may reduce the risk of ischemic stroke.

Sponsor:

1001

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IN VIVO ROLE OF THE NATURAL KILLER CELL RECEPTOR 2B4 (CD244) IN TUMOR REJECTION

Purpose: Immune system, innate as well as adaptive, plays an important role in the rejection of tumor cells. Cytotoxic T lymphocytes (CTL) of the adaptive immune system are antigen specific and require prior activation to kill cancer cells. Natural Killer (NK) cells, which belong to the innate immune system, on the other hand, are antigen-independent and can kill tumor cells immediately without prior simulation. This makes them the first line of defense against cancer. To understand this 'natural cytotoxicity' of NK cells we have identified several cell surface receptors involved in tumor rejection. 2B4 (CD244) is one such receptor that is expressed on all NK cells, monocytes, and some subsets of T cells. CD48 is its high affinity ligand and cross-linking 2B4 with anti-2B4 antibody in vitro causes activation of NK cells.

Methods: To study its physiological role we have generated, by gene targeting, mice deficient in the expression of this cell surface molecule. In this study we analyzed the lymphoid development and tumor rejection in these knock-out mice.

Results: The expression of cell surface markers on splenocytes of mice homozygous for the mutation in 2B4 (2B4-/-) is identical to wild-type mice. However, thymocytes from 2B4-/- mice have a reduction in the percentage of CD4+ and CD8+ cells. Double color staining of thymocytes for CD4 and CD8 reveals an increase in the immature CD4-/CD8- population and a reduction in the CD4+/CD8+. To investigate the in vivo role of 2B4 in tumor rejection, wild type and 2B4-/- mice were injected with CD48+ and CD48- metastatic B16 melanoma cells. Wild type mice rejected CD48+ melanoma poorly as compared to CD48- tumor cells suggesting that ligation of 2B4 by CD48 on melanoma cells inhibits NK function. In keeping with this, male 2B4-/- mice showed enhanced ability to reject CD48+ melanoma cells. However, female 2B4-/- mice poorly rejected both CD48+ and CD48- melanoma cells revealing a gender specific and CD48 independent defect in mice lacking 2B4.

Conclusions: Our results suggest that 2B4 is involved in the normal development of T cells. It appears that 2B4 might have a gender specific role in the immune system. Moreover, 2B4-CD48 interaction inhibits NK cells indicating that blocking 2B4-CD48 interaction may be a useful approach to reduce the metastasis of cancer.

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1002

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CHARACTERIZATION OF A NOVEL RECEPTOR CS1 IN HUMAN LYMPHOCYTES

Purpose: Immune cell functions are regulated by surface receptors and their interactions with ligands. While natural killer (NK) cells mediate innate immune response, B and T lymphocytes mediate the adaptive response. In order to decipher the mechanisms of regulation of the immune system, we have identified a novel receptor, CS1. CS1 is a member of the immunoglobulin superfamily (IgSF) and is expressed on NK, B and T cells. We have previously shown that CS1 is a self-ligand and homophilic interaction of CS1 regulates NK cell cytolytic activity. The cytoplasmic domain of CS1 contains immunoreceptor tyrosine-based switch motifs (ITSM), to which the signaling adaptor molecule SAP (SH2D1A), the defective gene in the X-linked lymphoproliferative disease (XLPD), binds and regulates immune cell function.

Methods: Recently, we have identified a novel splice variant of CS1 (CS1-S), which lacks ITSM motifs in the cytoplasmic domain. Human NK cells expressed mRNAs for both wild type CS1 (CS1-L) and CS1-S and their expression level remained steady upon various stimulations. However, human B cells express only mRNA for CS1-L. Here we inverstigated the function of each isoform in NK cells and B cells. cDNAs for CS1-L and CS1-S were transfected into the rat NK cell line RNK-16.

Results: CS1-L was able to mediate redirect cytotoxicity of P815 target cells in the presence of monoclonal antibody against CS1 suggesting that CS1-L is an activating receptor whereas, CS1-S showed no effect on the cytolytic function. Interestingly, SAP associated with unstimulated CS1-L and dissociated upon pervanadate stimulation. These results indicate that CS1-L and CS1-S may differentially regulate human NK cell functions. Freshly isolated B cells expressed only CS1-L isoform, which was rapidly upregulated upon activation. Importantly, monoclonal antibody against CS1 induced proliferation of activated B cells.

Conclusions: The results indictae that the novel receptor CS1 may play an important role in innate and adaptive immune responses.

Sponsor: *NIH grant CA85753*

1004

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TRANSCRIPTIONAL REGULATION OF 2B4 GENE IN HUMAN NATURAL KILLER CELLS

Purpose: The innate immune system plays an important role in the elimination of virally infected and transformed cells. Natural Killer (NK) cells belong to the innate immune system. Since, NK cells can kill target cells in an antigen-independent manner without prior activation they are considered the first line of defense in these situations. 2B4 (CD244), a member of the immuno-globulin superfamily, is a cell surface glycoprotein expressed on all NK cells, monocytes and some subsets of T cells. CD48 is its high affinity ligand and 2B4-CD48 interaction regulates NK functions. 2B4 has been shown to play a role in EBV, HIV, HSV and HHV8 infection and its expression is upregulated in these infections. To understand the specific upregulation of 2B4 in these viral infections we are studying the transcriptional regulation of the 2B4 gene.

Methods: In this study, we have analyzed the upstream positive regulatory region (-1151 to -704) of the 2B4 promoter using DNA footprinting, electrophoretic mobility shift assays, mutational analysis and luciferase reporter assays.

Results: A binding site for human NK cell nuclear protein was identified close to -945 nucleotide of the 2B4 promoter by DNA footprinting and electrophoretic mobility shift assay (EMSA). Mutation analysis showed that the nuclear factor bound between -955 and -945 nucleotides. Reporter assays in YT cells (a human NK cell line) indicate that the protein binding site is functionally active and upregulates transcription of the 2B4 gene. For functional activity, the -945 cis-acting element requires an AP1 site (-106 to -100). Additionally, we have found that the -945 and the AP1 cis-acting elements in the human 2B4 promoter function in a cell specific manner.

Conclusions: We have identified a novel cis-acting element within the human 2B4 promoter that functions in a cell specific manner.

Sponsor: *NIH grant CA85753*

1005

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INTERLEUKIN-4 DAMPENS METHACHOLINE-INDUCED BRONCHIAL HYPERRESPONSIVENESS DURING PULMONARY MYCOPLASMA INFECTION

Purpose: This study evaluates the role of IL-4 during methacholine-induced BHR during mycoplasma pulmonary infection.

Methods: Utilizing IL-4 knockout (KO) mice, we followed methacholine-induced BHR during M. pulmonis infection using whole-body plethysmography. We infected BALB/c and IL-4 KO mice with M. pulmonis, and then monitored Penh scores before and after methacholine inhalation with whole-body plethysmography.

Results: IL-4 KO mice showed no difference in histopathology of the lungs before or after mycoplasma infection when compared to BALB/c mice. IL-4 KO mice showed no difference in airway obstruction during mycoplasma infection when compared to BALB/c control mice, as both had increased airway obstruction from days 7 to 21 post-infection. However, IL-4 KO mice had significantly higher methacholine induced BHR during mycoplasma respiratory infection when compared to BALB/c mice. Uninfected mice showed no difference in methacholine-induced BHR between strains.

Conclusions: The loss of IL-4 led to exacerbated BHR during mycoplasma respiratory infection. This demonstrates that mycoplasma induced BHR is not IL-4 mediated, in fact IL-4 dampens this methacholine-induced BHR during mycoplasma disease.

Sponsor:

1006

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STRUCTURAL BASIS OF NK CELL ACTIVATION: IDENTIFICATION OF THE FUNCTIONAL AMINO ACID RESIDUES INVOLVED IN 2B4 -CD48 INTERACTION

Purpose: Natural killer (NK) cells are cells of the immune system that form the first line of defense against cancer and infectious diseases. NK cell cytolytic function is controlled by multiple receptor-ligand interactions. These receptors can transmit either positive or negative signals and belong to the lectin superfamily or immunoglobulin superfamily (IgSF). 2B4 (CD244), a member of the CD2 subset of the immunoglobulin superfamily, is the high affinity ligand for CD48. 2B4/CD48 interaction plays a vital role in the immune system by regulating NK and T lymphocyte functions. 2B4/CD48 interaction enhances non-MHC-restricted cytotoxicity in NK cells whereas in T cells it augments antigen-specific CTL lysis of specific targets. Defective signaling resulting from 2B4/CD48 interaction, due to a genetic defect in the adapter molecule SAP/SH2D1A has been implicated in the pathogenesis of X-linked lymphoproliferative disease.

Methods: To determine the structural basis for 2B4/CD48 interaction, selected amino acid residues in the variable (V) domain of the human 2B4 were mutated by site-directed mutagenesis. Following transient expression of these mutants in B16F10 melanoma cells their interaction with soluble CD48-IgG fusion protein was assessed by flow cytometry.

Results: We have identified amino acid residues in the extracellular domain of human 2B4 that are involved in interacting with CD48. Our data further demonstrate that Lys68 and Glu70 in the V domain of human 2B4 are essential for 2B4/CD48 interaction.

Conclusions: The present study has implication in designing small molecules that could block/modulate NK and T cell functions resulting from 2B4/CD48 interaction. (Research supported by NIH grant CA85753).

Sponsor: *NIH*

1007

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NK CELLS PLAY A ROLE IN THE GENERATION OF RESISTANCE AGAINST MYCOPLASMA

Purpose: The immune system contributes to both pathogenesis and protection in mycoplasma respiratory disease. A complex regulatory balance between the detrimental and beneficial effects of the immunity decides the course of the mycoplasma infection. The purpose of this study was to identify the impact of Natural Killer (NK) cells on the development of beneficial or detrimental adaptive immune responses.

Methods: BALB/c mice were used in these studies. The three groups tested were Control, Immunized and NK cells depleted prior to immunization. Mice were depleted, one day prior to each immunization, of NK cells by anti-asialo GM1 antibody treatment. Mice were immunized on day 1 and boosted on day 7 with mycoplasma membrane antigen. On day 14, sera were collected by retro-orbital bleed, and mice were challenged with 105 colony forming units (CFU) of mycoplasma. Sera were collected by retro-orbital bleed, on day 14 post-infection, and mice were euthanized. CFU were determined in lungs and nasal passages. Mycoplasma-specific antibody responses (IgA, IgM and IgG), in sera, were determined by endpoint titer ELISA.

Results: There was a significant decrease in the CFU isolated from lungs and nasal passages of the NK cell- depleted, immunized mice when compared to control and immunized mice. Prior to infection, there were no significant differences in antibody titer between the immunized groups. After infection, too, NK cell depletion did not affect the generation of mycoplasma specific antibody responses in immunized mice. However, the antibody titers for IgG and IgM were found to have significantly increased in NK cell-depleted immunized mice when compared to the unimmunized mice, both before and after infection.

Conclusions: NK cells have a detrimental impact on the development of adaptive immune responses in the clearance of the mycoplasma organisms from lungs and nasal passages. However, the mechanisms of adaptive immune responses that affect the clearance remain undefined.

Sponsor:

1008

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EPITHELIAL CELL RECOGNITION OF MYCOPLASMA

Purpose: The broad long-term objective of this project is to determine the molecular mechanisms central to the generation of the immune responses in mycoplasma respiratory disease. The specific aims of the study are as follows 1) Determine if the membrane components of M. pneumoniae activate or damage human bronchoepithelial cells and is this dependent on adhesion 2) Determine if M. pneumoniae membrane components stimulate human bronchoepithelial cells (BEC) through toll-like receptor (TLR) dependent mechanisms.

Methods: The study utilizes a series of co-culture experiments in which a primary human bronchoepithelial cell line is co-cultured with the membrane components (whole organism, membrane fraction, lipoproteins) of M. pneumoniae. To determine the stimulatory activity induced, untreated and pre-treated cells are harvested and primary transcript levels of selected cytokines and toll-like receptors are quantitated with RT-PCR and Super Array technology. Following, the conditioned medium (CM) is analyzed for its content of cytokines by an enzyme-linked immunosorbent assay (ELISA) or the luminex array system. To determine the necessity of adhesion, the stimulatory activity of a nonadherent mutant of M. pneumoniae (III-4) will be characterized as described above. To determine the utility of TLR in the stimulation of our BEC, we first identify the repertoire of TLR on our BEC cells with immunofluorescent staining and by monitoring primary transcripts as described previously.

Results:

Conclusions: Currently, we are determining the TLR responsible for innate recognition of mycoplasma. Consistent with expectations, current reports suggest the role of TLR family members in determing the type (TH1 vs. TH2) of immune responses generated in response to mycoplasma infection.

Sponsor:

1009

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COMPLICATIONS OF WEST NILE VIRUS SEEN AT UNTHSC IN 2003

Purpose: To report on neuroinvasive West Nile virus (WNV) infections seen at UNTHSC in 2003, and to compare this data with state and national trends. Based on prior seroepidemiological surveys, the incidence of neurologic complications has been described as less than 1% of WNV infections.

Methods: We describe the clinical presentation, laboratory data, and neurologic complications of 3 cases of WNV occurring in 2003, including the first report (Index case) of West Nile virus Meningoencephalitis (WNVME) in Tarrant County.
West Nile virus surveillance data was accessed from web sites for the Texas Department of Health (TDH), Zoonosis Control Division, and the Centers for Disease Control and Prevention (CDC) via ArboNet, the national electronic surveillance system for arboviral infections.

Results: From July to September, 3 patients (age 47-78) had symptoms of an acute febrile illness with mental status change, meningeal signs, and associated dyskinesias, vertigo, weakness, and ataxia. Lab analysis showed leukocytosis, hyponatremia, and CSF pleocytosis in all cases. West Nile virus was confirmed with IgM antibody (IgM MAC-ELISA) on CSF or sera with acute and convalescent titers.
In 2003 Tarrant County ranked 5th in Texas counties for WNV cases with 27.3% (6/22) having neurologic complications. Texas ranked 4th in the United States for WNV cases for 2003, but had the highest incidence for neurologic complications with 62.6% (415/663).
The total for national West Nile virus cases for 2003 was 9,175 with 30.0% (2773/9175) neurologic complications. This is 2.2 times the incidence rates for 2002 (9175/4156).

Conclusions: Cases of WNV infection seen at UNTHSC in 2003 are consistent with national trends which represent the largest arboviral epidemic of meningoencephalitis ever reported in the Western Hemisphere. Texas led all states in the incidence of neuroinvasive cases of WNV.
The data suggest that WNV has a degree of neurotropism which exceeds previous descriptions.
The most common complications of WNV are neurologic and the long-term morbidity is significant.

Sponsor: *n/a*

1010

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CYTOKNIE AND CHEMOKINE EXPRESSION PROFILING IN LUNGS REVEALED BY MICROARRAY FOLLOWING MYCOPLSMA PULMONIS INFECTION IN MICE

Purpose: Cytokines and chemokines are important mediator during the host□s immune/inflammatory response while combating invading pathogen. The purpose of these studies is to begin identifying pulmonary cytokines and chemokines produced in different mice strain at different time points in response to mycoplamsa respiratory disease.

Methods: C3H mice were intranasally inoculated with 1'105 colony forming units of Mycoplasma pulmonis (M. pulmonis) or broth culture medium (control). The mRNA expression of 96 cytokines, 67 chemokines and receptors were evaluated in lung tissues from BALB/c ,C3H/HeN and C57BL/6 mice at day 3, day 14 and day 28 post M. pulmonis infection using a membrane based cDNA microarray (SuperArray). Quantitative real-time RT-PCR was used to confirm the results from the array. The cell population infiltrated in lung response to M. pulmonis infections were observed by immuno-fluorescent staining (F4/80 staining macrophages, CD11c+ staining dendritic cells, B220+ staining B cells, CD4 and CD8 staining CD4+ T cell or CD8+ T cells) and H&E staining to observe neutrophills.

Results: C3H/HeN and BALB/c mice have very similar pattern of cytokine, chemokine and receptor changes. There were no consistent changes of any genes on day 3 post M. pulmonis infection. Cytokine IL-1a, IL-1betta, IL-6, IL-10, TNF-alpha, IFN-gamma; chemokine and receptors :CXCR-5, CXCR2, CCR-1, Pumag, MCP-5, MIP-3a, MIP-1b, IP-10, SCYB-11, BLC/BCA-1, MIP-2, MIG displayed two more fold increase both on day 14 and day 28 post infection. The only cytokine showed increase in C57BL/6 mice were IL-1beta. The infiltration of macrophages, dendritic cells, B cells and neutrophills were highly increased in the lung of M. pulmonis infected mice at day 14. CD4+ T cells were predominantly infiltrated into lung after infection. There is no significant alteration of CD8+ cells in the lung.

Conclusions: Both C3H/HeN and BALB/c mice are M. pulmonis sensitive strain, which have very similar pattern of cytokine, chemokine and receptor changes. C57BL/6 mice are M. pulmonis infection resistant strain showed very few cytokines alterations. We had an overall impression that altered cytokines were proinflammatory cytokines, those altered chemokine and receptors were associated with chemoattracting macrophages, immature dendritic cells, B cells, T cells, especially neutrophills into the lung response to M. pulmonis infection.

Sponsor:

1011

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EXPRESSION AND FUNCTIONAL ROLE OF THE LLT1 RECEPTOR ON LEUKOCYTES

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. This same study found that 100% of monocytes express LLT1, whereas LLT1 is expressed only on 5-10% of NK cells. A recent report shows that LLT1 is expressed on osteoclasts and it inhibits their formation and function. We hypothesize that LLT1 may function as an immune modulator, possibly linking a regulatory feedback loop between activated NK cells and macrophages.

Methods: 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: 100% of unstimulated U937 express LLT1. Activation of U937 cells by IFN-gamma induces a five-fold increase in LLT1 expression.

Conclusions: The expression and induction of LLT1 on U937 cells indicate that LLT1 may function as a link between innate and adaptive immune responses.

Sponsor: *NIH grant CA85753*

1200

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HEALTH PROMOTION IN SCHOOLS OF MUSIC

Purpose: The purpose of the Health Promotion in Schools of Music project is to develop health promotion materials for all college level music schools in the U.S.

Methods: Seek funding. Develop working teams of nationally recognized experts in four health areas (mental, neuromusculoskeletal, vocal, and mental health). Charge working teams with developing core content for health promotion media. Present and debate core content during national meeting of NASM accredited music school faculty and administrators. Package, test, and refine computer-based deliverable.

Results: Funding has been secured from National Academy of Recording Arts & Sciences, National Endowment for the Arts, International Music Products Association, International Foundation for Music Research, and the Scott Foundation. Four working groups of nationally acclaimed researchers and scientists are developed including several from the NIH. Several national and international partnerships are established and facilitating communication between musicians, music teachers, and faculty across the country. Conference activities are planned for September 2004 in Fort Worth.

Conclusions: This project is not designed to conclude, but rather influence the subjective social norms in college music programs regarding occupational injuries associated with music. However, at the current stage of development, we can conclude that there is enormous desire to address medical problems associated with music performance and to facilitate growth in this area.

Sponsor: *NARAS, NEA, NAMM, IFMR, Scott*

1300

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DISTRIBUTION RATIO OF PKC PLAYS A ROLE IN ESTROGEN PROTECTION AGAINST ETHANOL WITHDRAWAL TOXICITY IN RATS

Purpose: Previously, we reported that 17beta-estradiol (E2) administration reduces neurobehavioral
damage during ethanol withdrawal (EW). In this study, we examined whether distribution ratio (membrane / cytosol activity) of PKC plays a role in estrogen protection against ethanol withdrawal toxicity in rats. Altered distribution ratio of PKC has been implicated in neurodegenerative disorders.

Methods: Ovariectomized rats implanted with E2 (EW/E2) or oil pellets (EW/Oil) received oral administration of chronic ethanol (7.5% wt./vol., 10 days) or control dextrin solution (Dextrin/Oil). At 0 or 7 hours of EW, rats were sacrificed for collection of the cerebellum. The brain homogenates were further processed for total PKC and PKC epsilon activity using an in vitro [g-32P]ATP phosphorylation assay and immunohistochemistry using a Western blot method.

Results: At both 0 and 7 hours of EW, the EW/Oil group showed enhanced total PKC and PKC epsilon
activity as compared to the control or the EW/E2 groups. Membrane / cytosol ratio of both total PKC and PKCe was also higher in the EW/Oil group than the control or the EW/E2 group.

Conclusions: These data suggest that estrogen protects against chronic ethanol/ EW toxicity in part by preventing activation and membrane translocation of PKC.

Sponsor: *NIH/NIAAA 013864*

1301

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ROLE OF THE GABA-A SYSTEM IN ESTROGEN-INDUCED PROTECTION AGAINST BRAIN LIPID PEROXIDATION IN ETHANOL-WITHDRAWN RATS

Purpose: Our previous studies have shown that 17 beta-estradiol (E2) treatment protects against cerebellar neuronal death and related motor deficits in ethanol-withdrawn rats, in part through the GABAergic system. In this study, we examined the effect of the GABA-A antagonist, bicuculline on the neuroprotective effect of E2 by assessing the oxidative marker, thiobarbituric acid reactive substances (TBARS) during ethanol-withdrawal (EW).

Methods: Ovariectomized animals implanted with E2 (EW/E2) or oil pellets (EW/Oil) received liquid ethanol (7.5% wt./vol.) or dextrin for 7days by gavage. The GABA-A antagonist bicuculline (1.25mg/kg) was administered (t.i.d. IP) for 4 days starting 3 days before the onset of EW. After test for overt EW signs at 7 hours of EW, one set of the animals was immediately sacrificed for collection of the cerebellum, the hippocampus and the cortex. The brain homogenates were further processed for thiobarbituric acid reacting substances (TBARS) assay to detect TBARS in the presence or absence of FeCl3. The other set of animals was tested for the latency to fall from a rotarod after 1 week of EW to assess motor capacity.

Results: EW/Oil animals had enhanced endogenous and FeCl3 stimulated TBARS levels in the cerebellum and hippocampus in a manner potentiated by bicuculline but inhibited by E2. Bicuculline when administered along with E2 counteracted the protective effect of E2. Pearson correlation coefficients indicated that the latency to fall from rotarod covaried with TBARS levels in the cerebellum and hippocampus.

Conclusions: These data suggest that E2 protects against lipid peroxidation in vulnerable brain areas of ethanol-withdrawn rats, in part through the GABAergic system.

Sponsor: *NIH/NIAAA013864*

1302

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COMPARISON OF THE NEUROPROTECTIVE PROPERTIES OF ESTROGEN COMPOUNDS WITH CORRESPONDING QUINOLS IN A TRANSFORMED MURINE HIPPOCAMPAL CELL LINE

Purpose: The neuroprotective effects of estrogens have been well documented, however there are negative side effects including reactive oxygen species (ROS) production during redox cycling and feminization. In order to find a way to reduce these side effects, we tested the neuroprotective effects of the quinols of several steroid compounds. The quinol of estrone has previously been shown to be non-feminizing and in addition, the conversion of a quinol back to the parent estrogen does not produce ROS and thus may function as pro-drugs for the active hormone.

Methods: We tested 17²-estradiol (E2), 17±-E2, estrone (E1), adamantyl E1, and 5,6,7,8-tetrahydro-2-naphthol (THN) along with the corresponding quinols. Studies were initiated by plating HT-22 (transformed mouse hippocampal) cells in 96-well plates using Dulbecco□s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS) and gentamycin. When cells reached approximately 25% confluence, they were co-treated with one of the above-mentioned compounds at doses ranging from 0.01-10¼M and with glutamate at concentrations of 0, 5, 10, and 20mM. After 24 hours of exposure, a cytoprotection assay was administered. This assay uses calcein AM, a cell-permeable dye, which is cleaved by cytosolic esterases to produce a green fluorescence which we measured on a BIO-TEK FL600 Microplate Fluorescence Reader at 485/20 nm excitation and 530/25 nm emission.

Results: All parent compounds exhibited neuroprotection to varying degrees at all glutamate doses as was expected, however only the quinols of E2 and adamantyl E1 were effective in preventing cell death although both quinols proved to be less efficacious than the parent compounds. The quinol of adamantyl E1 provided maximal protection at a higher concentration than its parent while E2 protected up to 1¼M, after which it produced toxicity. The studies of 17±-E2 quinol, estrone quinol, and THN quinol indicated that these compounds may be toxic to these particular brain cells.

Conclusions: In conclusion, the quinols of E2 and adamantyl E1 are effective in providing neuroprotection in HT-22 cells when co-treated with glutamate, although to a lesser extent than the parent compounds, while 17±-E2 quinol, estrone quinol, and THN quinol seem ineffective. Further investigation must be done to determine optimal conditions for neuroprotection using quinols. Current research includes pre-treating HT-22 cells with the quinols before administering a toxic insult.

Sponsor:

1303

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BEHAVIORAL SENSITIZATION TO COCAINE AND METHAMPHETAMINE IN SWISS-WEBSTER MICE

Purpose: It is known that repeated use of Psychostimulant medications result in Sensitization,an increase in behavioral responsiveness.The purpose of these studies was to determine the influence of dose, context of drug exposure, Psychostimulant drug, on the behavioral expression of sensitization.

Methods: 240 Male Swiss webster mice were randomly assigned in groups of Ten. Psychomotor stimulant effects were assessed utilizing a Digiscan activity monitor. In a drug pre-exposure phase, mice in context dependent condition received daily injections(IP) of either (5,10,20 and 30 mg/kg) cocaine or (0.25,0.5,1 or 2 mg/kg) methamphetamine in the test apparatus(paired group). Mice in the context independent exposure condition received the same dosing regimen in their home cages (unpaired group). A control group received an injection of 0.9% saline. Following Pre exposure, mice remained undisturbed for an other 7 days (exposure test interval) prior to a challenge test with either cocaine (0,3,10,30 and 56 mg/kg)or methamphetamine(0,0.5,1,2 and 4 mg/kg) administered according to cumulative dosing regimen.Overall assessment of Sensitization was done by first pre-exposing mice to Methamphetamine or Cocaine or Placebo for 5 days, waiting for 7 days and then challenging the mice on 13th day to determine the effect of pre-exposure.

Results: A Dose related context dependent effect was observed in cocaine and Methamphetamine treated mice. This was seen at lower doses. 10 and 20 mg/kg for cocaine and 0.25 and 0.5 mg/kg for Methamphetamine.

Conclusions: Psychostimulants produced a dose dependent response to sensitization.
Context dependent sensitization was predominant,(compared to context independent)and was at a lower dose.
Cocaine and Methamphetamine showed no Qualitative differences in the amount of sensitization produced.

Sponsor: *NIDA*

1304

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ALPHA SYNUCLEIN EXPRESSION AND DOPAMINE TRANSPORTER BINDING FOLLOWING CONTINUOUS CHRONIC COCAINE ADMINISTRATION

Purpose: Alpha synuclein is a synaptic protein which may play a role in various neurodegenerative diseases. While the function of the protein is unknown it was previously reported that alpha synuclein can bind to and alter function of the dopamine transporter and is upregulated in the sunstantia nigra and VTA of human cocaine addicts. The purpose of this experiment was to evaluate changes in alpha synuclein expression and dopamine transporter binding in the VTA and substantia nigra following chronic cocaine administration.

Methods: Male Sprague Dawly rats were given 40mg/kg/day cocaine or saline for 14 days using osmotic mini pumps. The subjects were withdrawn from the treatment for 7 days, then sacrificed and the VTA and Substantia nigra were removed. The brain regions were then exposed to saturation binding assays using [125I]RTI-121 to measure dopamine transporter binding and analyzed for alpha synuclein expression using western blotting methods.

Results: Continuous chronic cocaine administration decreased alpha synuclein expression in the VTA and Substantia nigra while increasing dopamine transporter binding in these areas.

Conclusions: These data indicate that alpha synuclein expression is inversely related to DAT concentration and the behavioral expression of tolerance to chronic cocaine administration. Alterations in alpha synuclein expression and dopamine transporter number could be important for the induction of tolerance.

Sponsor:

1305

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EFFECT OF LONG-TERM COENZYME Q10 SUPPLEMENTATION ON LONGEVITY AND ON PSYCHOMOTOR AND COGNITIVE FUNCTIONS IN YOUNG, ADULT AND OLD MICE

Purpose: The goal of this study was to determine whether long-term Coenzyme Q10 (CoQ10) supplementation has a beneficial effect on psychomotor and cognitive functions in young, adult or old mice and whether it has the potential to increase life-span in mice

Methods: Beginning at four months of age, separate groups of mice were fed a control diet or diets supplemented with low or high concentrations of CoQ10. The low and high supplemented diets yielded daily CoQ10 intakes of approximately 148- and 654 mg/kg, respectively. Separate groups of these mice received a behavioral test battery after 3, 11 or 21 months on each diet, when they were 7, 15 or 24 months of age. A separate cohort of mice was maintained to observe mortality under the two different treatments.

Results: Results from the behavioral test battery indicated that supplementation did not consistently retard age-associated losses of cognitive or psychomotor function. On the other hand, the longevity study revealed a delay in the mortality of the low CoQ group but not of the high CoQ group when compared to the controls.

Conclusions: The results of this study suggest that long-term supplementation with coenzyme Q10 may retard mortality if taken at a relatively low dose; however it does not seem to delay age-related declines of psychomotor and cognitive function.(supported by NIH/NIA, RO1 AG 13563).

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1306

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ESTRATRIENE NEUROPROTECTION THROUGH ANTIOXIDANT, NON-ESTROGEN RECEPTOR MEDIATED MECHANISMS.

Purpose: Estrogens possess neuroprotective properties. Postmenopausal estrogen-replacement therapy has been shown to prevent or delay the onset of Alzheimer□s disease. In light of the fact that oxidative stress is implicated in many neurodegenerative diseases, it is reasonable to consider that estrogens exert their neuroprotective actions via an antioxidant mechanism.

Methods: The structure-activity relationship (SAR) was determined using a model based on oxidative-stress cytotoxicities induced by glutamic and iodoacetic acids in a murine hippocampal cell line. We assessed antioxidant potential by determining estratrienes□ ability to inhibit iron-induced lipid peroxidation in rat brain homogenates. We also determined whether estratrienes could bind estrogen receptor alpha and estrogen receptor beta by competition binding assays involving an enzyme fragment complementation method.

Results: We confirmed the role of a phenolic A-ring for neuroprotection and expanded these findings by showing that modifications made to the estradiol pharmacophore that increases phenoxy radical stability increases neuroprotective potency. We extended that relationship in a lipid-based antioxidant model and found that potent neuroprotectants were also able to inhibit iron-induced lipid peroxidation. Further, we found that these potent antioxidant protectants did not bind to estrogen receptors. Not only was there a lack of correlation between binding and neuroprotection, but there was a slight negative correlation in the affinity of the ligand for the estrogen receptor and its ability to protect against oxidative stress.

Conclusions: In conclusion, the antioxidant capacity of estratrienes protects neuronal cells against oxidative stress in a manner that is not mediated by classical estrogen receptor mechanisms but through its ability to prevent lipid oxidative stress events.

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1307

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ETHANOL-INDUCED NEUROTOXICITY IS ENHANCED IN PRIMARY CULTURES FROM ALPHA7 NICOTINIC RECEPTOR NULL MUTANT MICE

Purpose: Previous research from our laboratory and the laboratories of others have demonstrated that nicotinic agonists are protective against a variety of neuronal insults in both in vivo and in vitro model systems. Many of these studies have suggested a role of alpha7 nicotinic cholinergic receptors (nAChRs) in mediating the neuroprotective actions of nicotinic agonists. Of relevance to the present study, one neurotoxicant against which nicotinic agonists protect is ethanol, an agent that has also been reported to have an inhibitory effect on alpha7 nAChR function. In the studies presented here, ethanol-induced neurotoxicity was assessed in primary neuron-enriched cultures of cerebral cortical tissue derived from alpha7 null mutant (knock-out; -/-), heterozygous (+/-) and wild type (+/+) mice (C57BL/6 background). In alpha7 null mutant mice, there is no expression of alpha7 nAChRs and the alpha7 expression of heterozygous mice is approximately half that of wild type mice.

Methods: Tissues were collected and cultured on day of birth in DMEM (containing 20% Horse Serum) in 96 well plates. On Day 2, the mitotic inhibitor, Ara-C (10nM), was added and ~75% of the media was exchanged with fresh media 2 days later. Media was again changed every 3-4 days until ethanol treatments were initiated after cells were in culture for 2 weeks. Ethanol (0-150 mM) was added to the media in a fashion that its concentration remained stable for a 4 day treatment period. Cellular viability was then assessed using either the MTT or Calcein-AM assays.

Results: In both assay systems, ethanol was neurotoxic in a concentration dependent fashion and alpha7 null mutation enhanced this toxicity in a gene dosage related fashion.

Conclusions: These data provide further evidence of the putative role of alpha7 nAChRs in modulating ethanol-induced neurotoxicity.

Sponsor: *AA11597, AA09585 and the UNTHSC Tobacco Research Fund*

1308

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EFFECTS OF SEX STEROIDS ON STROKE

Purpose: The present study was to determine the effects of sex steroids on stroke

Methods: The effects of estrogen and testosterone on stroke were determined in both in vitro and in vivo model. For the in vivo studies, both subarachnoid hemorrhagic (SAH) and ischemic stroke model were set up to determine the effects of sex steroids. Immunocytochemistry, immunoblot as well as mass spectrometry were used to demonstrated the localization of estroge receptor beta (ERbeta).

Results: Our studies indicated that neuroprotective effects of E2 exert in both ischemic and hemorrhagic stroke. In our SAH model, E2 reduced secondary ischemic damage and mortality of SAH. These effects were not associated with the change of the clot volume in SAH. The neuroprotective effects of estrogens were not only seen in the pre-treatment paradigms. E2 exerted neuroprotective effects when administered after ischemia, with a therapeutic window in a permanent focal cerebral ischemia model of about 3 hours. This effect of estradiol was associated with no immediate change on blood flow, but with a delayed increasing in CBF. Further, our study indicated that a non-ER-binding analogue possessed both neuroprotective and vasoactive effects, which suggests that both the neuroprotective and vasoactive effects of estrogens are receptor-independent. This molecule also offers the possibility of clinical application for stroke without the side effects of estrogens. We used immunocytochemistry, immunoblot and mass spectrometry to demonstrate that ERb is localized to mitochondria. Our data established this ERb localization in a variety of cell types, suggesting that ERbeta is not a nuclear receptor, which was thought to mediate the genomic function of estrogens. In contrast to estrogens, testosterone increased neuronal toxicity and exacerbated cerebral ischemia-reperfusion injury. Further, our study indicated that stress induced testosterone reduction contributes to cerebral ischemia tolerance against ischemia reperfusion injury.

Conclusions: These results suggest that sex differences in outcome after stroke may resulted from both the protective effects of estrogens and the damaging effects of testosterone. Further, the interaction of testosterone and crebral ischemia tolerance provide the first in vivo evidences for a neuroendocrine mechanism for the cerebral preconditioning in males.

Sponsor:

1309

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ROLE OF PROTEIN PHOSPHATASES IN ESTROGEN MEDIATED NEUROPROTECTION

Purpose: The signaling pathways that mediate neuroprotective and neurodegenerative effects are extremely complex. A fine balance between phosphorylation and deposphorylation exists to maintain normal signaling. Protein phosphatases are essential modulators of protein kinases as well as direct effectors. Among the cellular processes regulated by protein phosphatases, oxidative stress induced cell death is of particular interest. We have previously shown that 17b-estradiol and its analogs have anti-oxidative properties. The purpose of this study was to delineate the mechanism by which estrogens are neuroprotective against oxidative stress.

Methods: HT-22 murine mouse hippocampal and C6 rat glioma cells were exposed to several different phophatase inhibitors at various concentrations in the presence or absence of 17 beta-estradiol and/or glutamate. Cell viability was measured by Calcein AM, a nonfluorescent, electrically neutral nonpolar analog of fluorescein diacetate, which passively crosses cell membranes and is cleaved to a fluorescent derivative by nonspecific intracellular esterases. Once cleaved in viable cells, the resultant fluorescent salts are retained by intact cell membranes.

Results: Okadaic acid and calyculin A, non-specific serine/threonine protein phosphatase inhibitors at various IC50, caused a dose-dependent decrease in cell viability in both HT-22 and C6-glioma cells. 17 beta-estradiol did not show protection against either okadaic acid or calyculin A in these cells. Moreover, in the presence of these serine/threonine protein phosphatase inhibitors, 17 beta-estradiol failed to protect against glutamate toxicity. Sodium orthovanadate, a general/broad specificity inhibitor of protein tyrosine phosphatases as well as alkaline phosphatases, also caused a dose-dependent decrease in cell viability in HT-22 and C6-glioma cells. Neither co-treatment nor pretreatment with 17 beta-estradiol prevented the dose dependent decrease in cell viability. In the presence of sodium orthovanadate, 17 beta-estradiol did not confer neuroprotection against glutamate toxicity.

Conclusions: These results suggest that 17 beta-estradiol may be protecting cells against glutamate induced oxidative stress by activating protein phosphatases. Further studies are needed to determine the specific protein phosphatases involved in 17b-estradiol neuroprotection against oxidative stress. (Supported by AG 10465 and AG 22550)

Sponsor:

1310

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ESTROGEN PROTECTS NITRIC OXIDE DONOR-INDUCED APOPTOSIS OF HT-22 CELLS THROUGH ERK INHIBITION

Purpose: Estrogens are sex hormones as well as neuroprotectants in age-related disorders such as Alzheimer's disease. Nitric oxide (NO) is a signaling molecule which plays an important roles in neuronal injury associated with ischemia, neurodegenerative disease, and excitotoxicity. We have previously shown that 17b-estradiol and its analogs are neuroprotective against oxidative stress. Here we show that 17beta-estradiol can protect against NO-induced apoptosis of HT-22 cells (a murine mouse hippocampal cell line) and NO-induced apoptosis of HT-22 cells is mediated by an extracellular signal-regulated kinase (ERK) pathway.

Methods: HT-22 cells were pretreated with kinase inhibitors for 1 hr before insult with sodium nitroprusside (SNP), a NO donor. Cell viability was measured by Calcein AM, a nonfluorescent, electrically neutral nonpolar analog of fluorescein diacetate, which passively crosses cell membranes and is cleaved to a fluorescent derivative by nonspecific intracellular esterases. Once cleaved in viable cells, the resultant fluorescent salts are retained by intact cell membranes.

Results: Treatment with SNP (100uM to 1uM) caused a dose-dependent decrease in cell viability. Inhibition of p38 MAP kinase by SB203580 (20uM) did not protect HT-22 cells against NO-induced cell death, whereas inhibition of MEK with U0126 (1 or20 uM) was protective.

Conclusions: These results suggest that NO-induced apoptosis of HT-22 is mediated by ERK activation and 17beta-estradiol can protect against NO-induced apoptosis of HT-22 by a mechanism involving inhibition of ERK activation.

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1311

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PROGESTERONE MODULATES INOSITOL 1,4,5-PHOSPHATE-MEDIATED CALCIUM SIGNALING IN BIPOLAR CELLS OF THE RODENT RETINA

Purpose: The function of nerve cells is critically determined by the regulation of their cytosolic calcium concentration. Inositol-1,4,5-trisphosphate receptors (IP3Rs), ligand-gated calcium channels on intracellular membranes, can modulate neuronal function substantially by releasing calcium from intracellular stores. Endogenous steroid hormones, their metabolic products, and chemical derivatives mediate protection against cellular damage in a variety of organs and cell types, preventing the consequences of acute insults and degenerative diseases that ultimately lead to cell death. Thus, evaluating the function of steroid hormones in the retina could not only reveal novel therapeutic strategies for treating retinal degeneration, but may represent an ideal system to study mechanism of disease progression and develop relevant treatments for neurodegenerative diseases. Here, we analyzed the effect of progesterone (P4) on IP3Rs expressed by mammalian rod bipolar cells, the first interneurons of the retina.

Methods: The expression and distribution of IP3Rs and progesterone receptors (PRs) in rod bipolar cells from rat and mouse retinas was analyzed using immunocytochemistry and specific antibodies. Functional effects were determined with single channel electrophysiology of IP3Rs and optical imaging of intracellular Ca2+ concentrations.

Results: IP3Rs and PRs were co-expressed and co-distributed in the somata of rod bipolar cells. Treatment of rod bipolar cells with P4 induced phosphorylation of IP3Rs, which was accompanied by an increase in IP3R channel activity and prolonged transients of elevated cytosolic Ca2+ concentrations. The kinetics of intracellular Ca2+ signals were spatio-temporally correlated with the kinetics of IP3R phosphorylation and the distribution of IP3Rs.

Conclusions: Our data indicate that steroid hormones can functionally influence the activity of intracellular Ca2+ release channels and thereby influence functional properties of neurons expressing such channels. Steroid hormones can induce intracellular Ca2+ transients directly without the involvement of other messenger substances. At the same time, steroid hormone activity can alter also the properties of neurons and influence subsequent responses to external stimuli.

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1312

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INTERACTIONS BETWEEN METABOTROPIC GLUTAMATE RECEPTOR TYPE 8 AND CALCIUM CHANNELS IS G-PROTEIN MEDIATED IN MOUSE ROD PHOTORECEPTORS.

Purpose: The aim of the study was to identify the mechanism of action that leads to changes in the cytosolic calcium concentration in mouse rod photoreceptors after activation of metabotropic glutamate receptor type 8 (mGluR8) by glutamate (1, 2). mGluR8 is the most recently discovered member of the metabotropic glutamate receptor family and its distribution and pharmacology indicates potentially major functions in the central nervous system (3).

Methods: Mouse retinae were dissected and dissociated to isolate photoreceptor cells. Cells were loaded with the fluorescent calcium indicator dye Fluo-3, and changes in the cytosolic calcium concentration were recorded by micro-spectrofluorimetry. Photoreceptor cells were incubated with the G-protein inhibitor, pertussis toxin (PTX). They were then stimulated with different mGluR8 specific agonists, L-2-amino-4-phosphonobutyrate, L-serine-O-phosphate, and L-glutamic acid.

Results: Photoreceptor cells that had been treated with the G-protein inhibitor PTX did not respond to mGluR8 agonists and their intracellular calcium levels remained unaltered.

Conclusions: PTX-sensitive G-protein activity is required to mediate the inhibitory effects of mGluR8 on plasma membrane calcium channels. This might be a common signaling mechanism for mGluR8 in other parts of the central nervous system.

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1313

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CDK5 IS INVOLVED IN THE CDK5 IS INVOLVED IN THE TRANSIENT CEREBRAL ISCHEMIA INDUCED ABERRANT NEURONAL CELL-CYCLE RE-ENTRY

Purpose: examined cell-cycle regulators involved in G1-S cell cycle progression after a transient focal cerebral ischemia, induced by middle cerebral artery (MCA) occlusion and its related signaling pathways

Methods: Middle Cerebral Artery (MCA) Occlusion, Immunoblotting, Immunohistochemistry, TUNEL Analysis, TTC stain, Brain delivery into ventricles, in vitro kinase assay, immuno and affinity precipitation

Results: In the cerebral frontoparietal cortex, we observed a marked induction of both Cyclin D1, a coactivator of Cyclin dependent kinase 2/4 (Cdk2/4), and proliferating cell nuclear antigen (PCNA), a DNA replication protein regulated by Cdk2/4. We further observed the phosphorylation of retinoblastoma (Rb) protein at Cdk phosphorylation sites in neurons in the ischemic cortex. We examined the incorporation of BrdU, a nucleotide analog that incorporates into newly synthesized DNA. Within 24 hr reperfusion after 60 min occlusion, substantial BrdU positive neurons were observed in the ischemic cortex. Further, Rb protein associates with Cdk5, and Cdk5 phosphorylates Rb protein in vivo and in vitro, Cdk5was induced during the ischemia/reperfusion process, potentially by the cleavage of its co-activator P35 to P25 by calpain.

Conclusions: These results indicate that transient ischemia/reperfusion cerebral damage induces signaling at the G-S cell cycle transition, and may constitute a critical step of the neuronal apoptotic pathway in ischemia/reperfusion induced neuronal damage, and Cdk5 deregulation is involved in this aberrant cell-cycle re-entry

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1314

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EFFECTS OF PKC ACTIVATION ON THE HUMAN GLYCINE RECEPTOR

Purpose: We are investigating the mechanism by which protein kinase C (PKC) regulates glycine receptor (GlyR) function. Specifically, we are testing the hypothesis that PKC elicits endocytosis of GlyR.

Methods: Immunofluorescence and confocal microscopy were utilized to monitor the surface expression of GlyR in living HEK 293 cells. Site-directed mutagenesis was utilized to mutate the PKC phosphorylation site located within the TM3-TM4 loop of the human alpha1 glycine receptor (hGlyRa1) subunit. HEK 293 cells were transiently transfected with either wild-type or mutant cDNA. Whole-cell patch clamp studies were performed to investigate the effects of constitutive endocytosis and PKC activation on glycine-gated currents at physiological temperature.

Results: Immunofluoresence studies conducted in living HEK 293 cells transfected with hGlyRa1 cDNA demonstrated that GlyR are endocytosed via constitutive and PKC-mediated pathways. Time-course studies indicated no significant change in the amplitudes of glycine-gated currents over time. Mutation of the PKC phosphorylation site of the hGlyRa1 did not attenuate the ability of PKC to irreversibly inhibit glycine-gated currents.

Conclusions: GlyR are internalized via constitutive and PKC-mediated endocytosis in a temperature-dependent manner. Maintenance of glycine-gated currents over time indicates that GlyR are being reinserted into the cell membrane at a rate comparable to their endocytosis. Preliminary data suggest the PKC phosphorylation site located within the TM3-TM4 loop is not involved in the regulation of PKC-mediated endocytosis.

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1400

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THE EFFECT OF OSTEOPATHIC MANIPULATIVE MEDICINE ON PULMONARY FUNCTION AND LUNG VOLUMES IN HEALTHY ADULTS.

Purpose: The application of OMM techniques for the diaphragm is based on the theory that correctly executed the physician can alter the mechanics of respiration. Treatment of the diaphragm will allow greater excursion of the diaphragm into the chest resulting in decreased residual volume (RV).

Methods: Study design consisted of two groups; treatment and sham treatment. A pulmonary function test (PFT) was administered to each subject upon admission to the study. Following a short rest period the subject received either an OMM treatment or a sham treatment. A post treatment series of PFTs were then administered to the subject.

Results: A Paired T test comparing pre and post values within each group showed that changes in RV, TLC, and ERV were significant. Univariate Analysis of Covariance between groups also showed that changes in RV were significant.

Conclusions: From this data we conclude that OMM treatments addressing diaphragm function are effective in altering the mechanics of respiration and particularly in decreasing residual volume.

Sponsor:

1501

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TEEN PREGNANCY: THE PHYSICIAN ASSITANT'S PERCEPTION OF THEIR ROLE IN PREVENTIVE COUNSELING

Purpose: This study intended to investigate the Physician Assistant□s (PA) attitudes of preventive counseling as a tool to curbing pregnancy rates, as well as the frequency by which they counsel adolescent and their families.

Methods: The survey was reviewed and approved by the UNTHSC IRB before any data was collected. A 21-question survey was sent via email to 951 licensed and clinically practicing PAs who have an e-mail address on file with Texas Academy of Physician Assistants. The survey was validated by presenting it to three practicing PAs and eliciting their feedback as to any ambiguous questions or phrases. The investigators sent out 951 emails. 240 emails were undeliverable, and 244 responded (return rate of 33%). The data from thirty surveys were excluded due to failure to complete the survey. The total valid survey was 214.

Results: The study found that: 1). Most PAs (86-99%) felt it was their responsibility to provide preventive medicine 2). Most PAs (72-89%) felt they were qualified to counsel adolescent patients and their families on pregnancy prevention; 3) However, only 17% of PAs reported that they counseled over 25% of their adolescent patient population on pregnancy prevention; 3). The characteristics of PAs who responded with greater counseling rates (more than 25 % of their adolescent patient population) were: in a federally underserved setting (34%; $p<.01$), in a primary care field (27%; $p<.01$), and in a setting they described as □mostly rural□ (27%; $p=.042$).

Conclusions: The results indicated that PAs who responded believed preventive counseling to be an effective way of curbing adolescent pregnancy rates. Respondents also believed they are responsible to counsel their adolescent patients and their families on pregnancy prevention. However, less than 20% of PAs frequently counseled their adolescent patients on pregnancy prevention. Those PAs who provided more frequent pregnancy prevention counseling to adolescent patients were primary care providers (83%). Future studies should focus on the apparent gap between the PA□s intentions and their actions.

Sponsor:

1502

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ATTITUDES OF PHYSICIAN ASSISTANTS TOWARD NUTRITION EDUCATION AND NUTRITION RELATED ISSUES ENCOUNTERED IN CLINICAL PRACTICE

Purpose: The purpose of this study was to investigate how often Physician Assistants (PA's) encounter patients with nutrition education or medical nutrition therapy needs, and to assess how comfortable the PA feels when attempting to address those needs.

Methods: A 26 question survey instrument was developed and e-mailed to 951 prospective participants. The survey was reviewed for content by three registered, licensed dietitians and reviewed for clarity by two advanced practice nurses and one public health specialist. E-mail addresses were obtained with permission from the Texas Association of Physician Assistants and the project was approved by the IRB at UNTHSC. Statistical analysis was performed using SPSS (11.0). Chi square was used to compare differences between sets of units of data.

Results: Seven hundred and eighteen PA's received the e-mail and 134 PA's responded (18%). Eighty percent of respondents reported that their patients had nutrition related inquiries at least once per day and sometimes up to 10 times per day. Most PA's who responded to the survey felt competent or very competent providing basic preventative nutrition counseling as well as disease specific nutrition counseling. PA's sense of competency had a significant correlation with their opinion of the nutrition education that they received in PA school ($p<.01$). Another important finding was that those PA's who felt more competent in their abilities spent more time discussing nutrition issues with their patients than did those who felt less competent ($p<.001$).

Conclusions: The results data may be helpful in determining curriculum needs in PA training so that PA's are well prepared providing nutrition therapy to their patients.

Sponsor:

1503

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THE PRACTICE ENVIRONMENT OF PHYSICIAN ASSISTANTS IN CARDIOLOGY

Purpose: To better understand the practice environment of Physician Assistants in a cardiology practice discipline, specifically clinical role and practice demographics, the Association of Physician Assistants in Cardiology conducted its first survey of Physician Assistants listing Cardiology as their primary discipline of practice.

Methods: In 2003, a convenient sample of 582 Physician Assistants who were identified by the 2003 AAPA census as listing cardiology as their primary practice discipline were mailed a 17-question survey and provided a self-stamped, return envelope. Descriptive statistics and Chi-Square test were applied to analyze all data returned.

Results: A total of 283 completed surveys were returned (49%). The typical length of time identified in the cardiology practice setting was 1- 5 years (59%). The primary practice setting most commonly identified was the combined noninvasive and invasive practice (68%). Physician Assistants were involved in both inpatient (52%) and outpatient (50%) care. Common clinical duties included admission history and physical examinations (87%), hospital rounds (82%) discharge summaries (78%), cardiology consults (73%), post-operative management (73%), outpatient management (71%), exercise stress testing (70%), nuclear stress testing (64%), and critical care management (60%). Invasive procedures PA's were performing included central line placement (14%), Swan-Ganz catheter placement (11%), intra-aortic balloon pump placement (11%), temporary pacemaker (10%), versus permanent pacemaker (4%), right heart catheterization (7%), and left heart catheterization (5%). More than half of respondents stated they were involved in clinical education of other health care professionals (58.6%) and 23.2% were involved in research.

Conclusions: The AAPA 2003 census notes that approximately 3% of Physician Assistants practice in a cardiology practice. The clinical roles of PA's in cardiology tend to be closely divided between outpatient and inpatient care. Clinical duties most commonly performed by PAs include admission history and physicals, discharge summaries, outpatient management, cardiology consults, postoperative management, critical care management, and exercise stress testing. In addition Physician Assistants are being utilized to perform invasive cardiac procedures.

Sponsor:

1603

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CORRELATION BETWEEN MECHANICAL AND ENZYMATIC EVENTS IN CONTRACTING SKELETAL MUSCLE FIBER

Purpose: The conventional hypothesis of muscle contraction postulates that the interaction between actin and myosin involves tight coupling between the power stroke and hydrolysis of ATP. However, some in vitro experiments suggested that hydrolysis of a single molecule of ATP caused multiple mechanical cycles.

Methods: To test whether the tight coupling is present in contracting muscle, we simultaneously followed mechanical and enzymatic events in a small population of cross-bridges of glycerinated rabbit psoas fibers. Such a small population behaves as a single cross-bridge when the contraction is initiated by a sudden release of caged ATP.

Results: Mechanical events were measured by changes of orientation of probes bound to the regulatory domain of myosin. Enzymatic events were simultaneously measured from the same cross-bridge population by the release of fluorescent ADP from the active site.

Conclusions: If the conventional view were true, ADP desorption would occur simultaneously with dissociation of cross-bridges from thin filaments and would be followed by cross-bridge rebinding to thin filaments. Such a sequence of events was indeed observed in contracting muscle fibers, suggesting that the mechanical and enzymatic events are tightly coupled in vivo. (NIH Grants R21CA9732 and R01AR048622 and Grant 000130-0008-2001 from the Texas Higher Education Coordinating Board)

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1604

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CHANGES IN MAMMALIAN CHROMATIN STRUCTURE AS A FUNCTION OF PROTEIN-POLY(ADP-RIBOSYL)ATION. SUSCEPTIBILITY OF INTERPHASE CHROMATIN TO ENZYMATIC DIGESTION WITH DEOXYRIBONUCLEASE I (DNASE I) AND MICROCOCCAL NUCLEASE (MNASE)

Purpose: To demonstrate that the poly(ADP-ribosyl)ation of proteins causes the relaxation of condensed interphase chromatin as determined by susceptibility to enzymatic digestion with Deoxyribonuclease I and Micrococcal Nuclease.

Methods: Mammalian chromatin was exposed to either DNAse I or MNase digestion as a function of the time of incubation and enzyme concentration. As a negative chromatin control, the same experiments were performed with calf thymus naked DNA. Endonuclease enzymatic reactions were stopped with loading buffer containing EDTA. Samples were subsequently run in 1.5% agarose gels and the oligonucleosomal electrophoretic migration patterns were compared after ethidium bromide staining of DNA.

Results: Endonuclease experiments were carried out with rat liver chromatin pre-incubated in the presence or absence of betaNAD+, the poly(ADP-ribosyl)ation substrate. We also used 1.0 mM benzamide, a competitive inhibitor of protein-poly(ADP-ribosyl)ation, to stop enzymatic modification, in the presence of 200 mM betaNAD+. Our electrophoretic observations demonstrated that the endonuclease digestion of interphase rat liver chromatin, with either DNAse I or MNase, was faster when proteins were covalently poly(ADP-ribosyl)ated for 30 min in the presence of betaNAD+ prior to internucleosomal degradation.

Conclusions: The covalent poly(ADP-ribosyl)ation of interphase chromatin proteins, especially histones, induces a more relaxed structure, rendering chromatin more sensitive to endonuclease digestion. Thus, our results are consistent with the conclusion that protein-poly(ADP-ribosyl)ation modulates chromatin structure and function.

Sponsor: *Department of Veterans Affairs*

1605

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THE 40 KDA CARBOXY-TERMINAL DOMAIN OF POLY(ADP-RIBOSE) POLYMERASE-1 FORMS CATALYTICALLY COMPETENT HOMO- AND HETERO-DIMERS IN THE ABSENCE OF DNA

Purpose: The purpose of this project was to determine the enzyme mechanism of the 40-kDa carboxy-terminal catalytic domain of PARP-1, a protein considered to be a □guardian angel□ of human genomic integrity.

Methods: State of the art methods of biochemistry and molecular biology were utilized in this project. These include, gene cloning, gene expression, protein purification by affinity chromatography, radio-enzyme assays, high resolution polyacrylamide gel electrophoresis, LDS-PAGE, autoradiography, physico-chemical and kinetic assays, and protein ultra-filtration.

Results: Homogeneously pure 40-kDa domain of PARP-1 synthesized covalent CD-poly(ADP-ribose) conjugates in the absence of DNA. Electrophoretic analysis of the ADP-ribose chain lengths generated indicated that recombinant CD was able to catalyze the initiation, elongation, and branching reactions of poly(ADP-ribose) synthesis, although at a 500-fold lower efficiency than wild-type PARP-1. Kinetic evaluation of poly(ADP-ribose) synthesis showed that the enzymatic activity of CD increased for up to 60 min in a time-dependent manner. Moreover, the rates of CD auto-poly(ADP-ribosyl)ation increased with second order kinetics as a function of the protein concentration with either betaNAD+ or 3□-dNAD+ as a substrate. The formation of catalytically competent CD-[PARP-1] heterodimers was also observed in specific ultra-filtration experiments.

Conclusions: We conclude that the 40-kDa carboxy terminus of PARP-1 forms catalytically competent homo- and hetero-dimers in the absence of DNA. Our results are also consistent with the conclusion that the auto-poly(ADP-ribosyl)ation reaction of the 40 kDa catalytic domain is intermolecular.

Sponsor: *NIH/NIGMS*

1700

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WEIGHT LOSS PRACTICES OF ADULT US JUDO COMPETITORS

Purpose: The purpose of this study was to determine weight loss practices of US judo competitors 18 years of age and older.

Methods: A 22 question survey was administered to competitors 18 years of age and older at a local judo tournament in North Central Texas in winter 2004. Survey items included demographic characteristics, cutting weight practices, and nutritional supplement use. Survey responses were anonymous, and participation was voluntary. The survey methodology was approved by the UNTHSC Institutional Review Board. Demographic characteristics were summarized using descriptive statistics. Linear regression models were used to determine if the length of time a competitor has practiced judo can predict the average amount of weight (in pounds) cut for a tournament and the most amount of weight (in pounds) ever cut for a tournament by gender.

Results: Forty-six of the 99 eligible adult competitors completed the survey resulting in a 46% response rate. .Of the 46 respondents, 28 (60.9%) responded yes to □Have you ever □cut weight□ for tournaments□. Of those having cut weight, 19 (67.9%) were male, the number of years practicing judo ranged from 1 to 25 (mean = 8.19), the average amount of weight (in pounds) cut for a tournament ranged from 2 to 15 (mean = 5.5), and the most weight (in pounds) ever cut for a tournament ranged from 2 to 22 (mean = 9.6).
For males, the number of years practicing judo was a significant predictor for both average pounds cut ($p = 0.043$) and most pounds cut ($p = 0.03$). For females, the number of years playing judo was not a significant predictor of either average pounds cut ($p = 0.408$) or most pounds cut ($p = 0.593$).

Conclusions: Cutting weight, or intentional rapid weight loss, is a common practice among judo competitors. This cross-sectional study of competitors at a local judo tournament demonstrates that approximately 61% of competitors engage in the practice and that for males the longer a competitor has been practicing judo, the more weight he is likely to have cut for a tournament. If primary care and sports medicine physicians are unaware of these practices, important diagnoses may be missed. The education of physicians and coaches about the prevalence and deleterious effects of these practices is essential.

Sponsor:

1701

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TOWARDS COMPUTATIONAL EPIDEMIOLOGY: APPLICATIONS IN EPIDEMIOLOGY AND COMPUTER SCIENCE

Purpose: Epidemiological studies often are based on spatially and/or temporally distributed information. The sources of data on which such studies are based are multi-factorial and must be carefully analyzed to account for bias, such as confounding and effect modification. One computational challenge is to combine the spatially and temporally distinct datasets. This necessitates a fundamental knowledge of database systems, data management, and data retrieval. Even if a comprehensive dataset, which may contain a multiplicity of individual studies, could be constructed, the extraction of relationships among the data constitutes a second computational challenge. The domain of Artificial Intelligence and Machine Learning has been successfully used in Bio-informatics and is likely to be a valuable tool for discovering relationships among epidemiological data. As the complexity of epidemiological information increases, it becomes imperative to apply efficient algorithms in these analyses. However, epidemiologists presently receive little or no training in the fundamentals of computer science, and computer scientists rarely have the requisite understanding of epidemiology, thus slowing the development of computational tools for epidemiological studies. Due to population growth and the impact of accelerated global transportation infrastructure, local health economies are strongly influenced by the global environment. As this trend continues, the domains of public health and epidemiology will play a dominate role in protecting our communities health. Epidemiological studies today are not just limited to small spatial domains, but often require a consideration of data from other regions in the world. In addition, the recent concern with infectious disease outbreaks, such as SARS, the threat of bio-terrorism events, and the need to protect citizens make it imperative to develop computational tools that help accelerate epidemiological surveillance and action.

Methods:

Results:

Conclusions: This presentation will briefly demonstrate the potential benefits and application of computational epidemiology in routine surveillance activities of on-going transmission of disease in a community.

Sponsor:

1702

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Scott McNabb, PhD, Centers for Disease Control and Prevention, Atlanta, GA

A STANDARDIZED TOOL FOR ASSESSING TB SURVEILLANCE AND ACTION PERFORMANCE AND COST

Purpose: The Centers for Disease Control recognize the need for standardized tools to evaluate tuberculosis surveillance, action, performance and cost. The University of North Texas Health Science Center in Fort Worth is tasked to produce this tool. We evaluate tuberculosis control programs with both economic and health outcome measures.

Methods: We describe a retrospective analysis of costs and program outcomes for 2003 within three Texas public health programs. We approach the analysis from both societal and public sector perspectives. This approach is broadly useful to allow programmatic and societal comparisons of cost-effectiveness of TB eradication efforts. We use counterfactual analysis to develop a reference case comparing effectiveness to the absence of a surveillance (public health) program and to no treatment. This approach confers the ability to judge the whole impact of a program. Sensitivity analysis aids in addressing uncertainty in patterns of demographics, epidemiology, and other issues. A base-case is derived using Markov modeling techniques. Study results will be presented in terms of both a standard metric of health intervention effectiveness and a TB disease specific metric. This allows both broad and narrow comparisons to be made, from among all health interventions to TB programs alone.

Results: Costs and outcomes of Tarrant County's tuberculosis control and surveillance program are presented in terms of cost per life years gained as well as tuberculosis cases averted.

Conclusions: Cost-effectiveness analysis is a valuable means to measure outcomes of tuberculosis control programs. This model is widely applicable for public health program evaluation in the United States.

Sponsor: *CDC*

1703

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SCREENING INTERNATIONAL STUDENTS FOR TUBERCULOSIS AT AN AMERICAN UNIVERSITY

Purpose: Nonimmigrant, international students entering most colleges and universities in the United States are not required to undergo screening for tuberculosis as part the immigration process. More than 50% of active cases in the United States are foreign-born individuals and most of them become active within 2 years of arrival. Controlling transmission from cases occurring on college campuses are difficult, and often labor and resource intensive.

Methods: In an effort to minimize the occurrence of active TB at a state university with an increasing international student population, the public health department in collaboration with the university's health services implemented a TB-screening policy. This policy requires tuberculin skin testing and chest x-rays for all newly enrolled international students.

Results: For the 2003/04 academic year, 779 international students from 59 different countries enrolled for classes. Three hundred and fifteen students (40%) screened had a positive skin test (greater than 10 mm induration). Of those, 3 students were identified smear negative culture positive tuberculosis, with 1 student with resistance to isoniazid, ethionamide, pyrazinamide, and streptomycin.

Conclusions: As the proportion of foreign-born cases in the United States increases, elimination of tuberculosis will depend on finding more proactive approaches to identifying potential cases. Requiring screening of nonimmigrant students at universities with an increased international population may increase case identification before they are symptomatic and contagious. This approach may be effective to reducing the local morbidity.

Sponsor:

1704

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TUBERCULOSIS TRANSMISSION DYNAMICS IN AN URBAN HOMELESS SHELTER

Purpose: Homelessness is one of the greatest risk factors for contracting tuberculosis (TB) in the United States. Current research suggests that homeless shelters may be common locations for tuberculosis transmission with urban communities. To our knowledge, no research exists on the dynamics of localized TB transmission that occurs within homeless shelters. This presentation examines the micro-geography of the distribution of TB cases and infection at a homeless shelter in Fort Worth, TX.

Methods: Earlier epidemiologic studies of tuberculosis cases identified one homeless shelter as the focal point for on-going transmission for the larger Fort Worth community. A targeted screening effort in this high-risk population was needed to reduce on-going transmission. Data was prospectively collected through interviews of TB patients during routine clinical practice and surveillance at the Tarrant County Health Department. Demographic characteristics were summarized using descriptive statistics. Multiple linear regression was used to estimate the risk of infection and disease based on bed location and duration of stay at the shelter.

Results: The results suggest that TB risk is not uniformly distributed with the shelter but depends on the location of the sleeping bed and duration and frequency of stay at the night shelter.

Conclusions: The information obtained from this study is important to understanding the social and physical dynamics of disease transmission within shelters. Understanding the risk factors associated with on-going transmission is important in the development of a more effective means of tuberculosis control. These data can be used to identify where transmission is most likely to occur and can be used to focus contact investigations. The implementation of geographic information systems in conjunction with surveillance data enables the identification of previously undetected transmission in a well-defined geographical setting.

Sponsor:

1705

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Y'ALL DOCTORS! I AIN'T FOR SURE I WANNA STAY HERE - A TEXAS PERSPECTIVE OF THE DISCHARGES AGAINST MEDICAL ADVICE

Purpose: This study was designed to profile the characteristics of patients who are discharged against medical advice in Texas hospitals between the years 2000-2002. In particular, emphasis was laid on the patient□s age, gender, race/ethnicity, source of pay/insurance, type of admission and length of stay and their roles in patients who are discharged against medical advice.

Methods: Hospital inpatient discharge data that is available as a Public Use Data File from the Texas Health Care Information Council was analyzed. All the three years data were pooled and analyses were done using descriptive statistics, frequencies and chi-square statistics through SPSS statistical program.

Results: A total of 50,574 patients were discharged against medical advice for the years 2000 to 2003. 0.365 % of the females discharged and 0.624% of the males discharged were against medical advice. Children belonging to the age group of 17 years and under comprised 5.9%, adults in the age group 18-44 comprised 40.8% and 65+ year age group comprised 19.7% of DAMA. 54% of the DAMA patients were White, 24.6% were of Hispanic origin and 16% were Blacks. Medicare DAMA patients comprised of 27.2%, self pay 23.4%, Medicaid 19%, Commercial insurance 19.7% and 10% were other sources of payment. The mean length of stay was 3.28 with a STD of 5.719. 42.6% of DAMA patients left the hospital in the first day, 19.2% in the second day, 12.2% in the third day and 91.8% of the patients left within 7 days.

Conclusions: The results from this research provide valuable information that can help physicians identify those patients who are at most risk for leaving hospitals against medical advice. This will allow them an opportunity to initiate preventive measures and perhaps design novel strategies to reduce attrition and improve the utilization of treatment resources. This in turn will benefit patients, physicians, payers and policy makers.

Sponsor:

1706

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DEVELOPING A STANDARDIZED EVALUATION TOOLKIT FOR TUBERCULOSIS CONTROL PROGRAMS

Purpose: As we move closer toward tuberculosis elimination, performance measurement and individual program evaluations will become increasingly important to target specific interventions for improved cost efficiency and performance. Surveillance activities are an important component of the elimination strategy. The need for an objective, simple, and standardized evaluation toolkit to uniformly measure both performance and efficiency of surveillance activities is necessary.

Methods: We used a community-based solution approach involving participatory research methods to enhance the promotion and development of an outcome based evaluation toolkit. Using a series of focus groups, composed of stakeholders from various professional levels within each control program, we developed a set of performance indicators. To measure performance in a standardized and objective fashion, the selection of indicators was matched to the core and support activities universally used by TB control programs. These indicators selected best reflect the validity, efficiency, practicality and feasibility in the local and regional environment in Texas

Results: Preliminary results of implementing the developed toolkit in North Central Texas will be presented.

Conclusions: This demonstration project serves as model for implementing a standardized, user-friendly evaluation toolkit. The use of three diverse departments in North Central Texas from urban and rural settings allows the created tool to be more generalizable to the United States as a whole, as it includes, high, moderate, and low incident programs at both the county and regional level.

Sponsor:

1707

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ACCULTURATION AND PSYCHOLOGICAL DISTRESS IN MEXICAN-AMERICAN HEALTH FAIR PARTICIPANTS

Purpose: This study was designed to determine the level of psychological well-being as it relates to acculturation for Mexican-Americans who participate in community health fairs.

Methods: Self-report questionnaires designed to assess psychological functioning and level of acculturation were offered as part of a health service booth at the Hispanic Health Fair in Fort Worth, Texas. Forty-three people identifying themselves as Mexican-Americans completed the questionnaire. Questionnaires were offered in Spanish or English. The Psychological Distress subtest from the Multidimensional Health Profile was utilized.

The acculturation measure is designed to provide an average score between 1 and 5, with 1 being the lowest level of acculturation and 5 being the most closely acculturated to the American culture. Due to the presence of a bimodal distribution, the data was split for comparison purposes; Low Acculturated (LA) and Moderately Acculturated (MA). Data was analyzed via F test, to determine group differences.

Results: Results of the psychological survey indicated a normal range of Overall Psychological Distress with an average standard score of 53.4. LA had a mean acculturation score of 1.42 and a mean psychological distress score of 58.0. MA had a mean acculturation score of 3.32 and a psychological distress score of 48.6. The overall degree of psychological distress was significantly higher for LA than MA, $F(1, 35) = 8.610$, $p = .006$. Analysis of psychological subscales determined a higher degree of anxiety for LA as compared to MA, $F(1, 35) = 6.901$, $p = .013$. The difference in level of depression between the two groups was in the same direction but not statistically significant.

Conclusions: Results of this study suggest that a mild, but clinically relevant degree of overall psychological distress is likely prevalent in Mexican Americans who are the least acculturated to the U.S. culture. Symptoms associated with anxiety are significantly higher for less acculturated individuals, while there is no substantial difference in depressive symptoms with regard to acculturation. These results underscore the importance of gaining knowledge about the needs and limitations faced by the sector of Mexican-Americans that are rarely seen within a traditional health service context. While the sample was small, these results point to a potential public health issue within the growing immigrant community that should be further investigated.

Sponsor:

1708

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HOPE FOR HEALTH, PREVENTION OF CHILDHOOD OBESITY.

Purpose: Childhood obesity has reached epidemic proportion and is directly attributed to physical inactivity and diet. Schools can play a key role in reversing this trend through comprehensive programs that promote healthy habits. The present study will be addressing the effect of launching and implementation of such a program, the □Health Centers□, to the elementary level classrooms. This community project was carried out by four students from Coppell Middle School North, under the guidance of Dr. Maya P. Nair.

Methods: The present study will be addressing the effect of launching and implementation of □Health Centers□, to the elementary level classrooms. A □Health Center□ is like any other study centers to learn the fundamentals. It provides a structured daily reinforcement about the importance of healthy eating, drinking, and being physically fit. A □Health Center□ will offer games, posters, flash cards, bookmarks with healthy quotations, a food pyramid, and physical activity pyramid.

Results: Pilot Study-The improvement in healthy habits for the group that receive a structural daily reinforcement through the □Health Centers□ about the importance of developing healthy habit is statistically significant ($p<0.05$).
In small group study also there is significant weight loss for the group with reinforcement and 90% parents agreed that they see improvement in their child□s healthy habits. In some cases even the parents getting benefited from the program as shown by the weight loss data.

Conclusions: Childhood obesity can be prevented by education, encouragement and daily reinforcement of healthy habits. The □Health Center□ designed by the team proved to be a key tool in improving the healthy lifestyle of our kids. Thus it will also help us to prevent childhood obesity.

Sponsor:

1709

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SPATIOTEMPORAL ANALYSIS OF ACCIDENTAL POISONING MORTALITY IN TEXAS (1980 - 2001): IMPLICATIONS FOR EMERGENCY PREPAREDNESS

Purpose: Various studies in the literature suggest the presence of spatial and temporal clusters in poisoning mortality and morbidity. However, many of the studies have examined almost exclusively excess mortality due to long-term exposure to specific agents and have not explicitly studied the possible persistence of mortality due to accidental poisoning across space and time. The objectives of this present study are twofold: one, to determine the existence of spatial and temporal clusters of accidental poisoning mortality in Texas; two, to determine if an association exists between accidental poisoning mortality and shortages of physicians specializing in emergency medicine

Methods: The Spatial Scan Statistic was employed to examine the geographic excess of mortality of poisoning by race in Texas counties between 1980 and 2001. The statistic was conducted with a scan window of a maximum of 90% of the study period and a spatial cluster size of 50% of the population at risk, and with a purely spatial option to verify whether the excess mortality persisted to the present decade. A map layer of the number of physicians in each county was placed on the clusters detected to evaluate the potential association.

Results: Two regions were identified with poisoning mortality excess in all populations: public health regions 2 and 3 from 1993-1998 at relative risk (RR) = 1.8 ($p=0.001$). Additionally, 2 clusters were detected for non-Hispanic Whites and Blacks, and another for Hispanics. Black males in 3 counties around Tarrant county had the longest excess mortality, between 1983-1998, RR=2.3, $p=0.001$). Hispanics in west Texas had the highest relative risks between 1985 and 1998 (RR=3.6, $p=0.01$). The spatial clusters correlate with health professional shortage areas

Conclusions: Spatiotemporal variations in poisoning mortality affected racial groups at varying levels. Several potential hot-spot clusters were detected, no evidence indicated a persistent spatiotemporal trend of excess mortality into the present decade. Black males in the Metroplex and Hispanics in West Texas carried the highest burden of mortality as evidenced by spatial concentration and temporal persistence. The results provide partial evidence for an association between areas with excess mortality and emergency medicine physician shortages

Sponsor:

1710

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ASSESSMENT OF PUBLIC HEALTH STUDENTS' ATTITUDES TOWARD TOBACCO CONTROL

Purpose: Public health professionals are usually the purveyors of effective strategies for tobacco control. However, information on public health professionals' attitudes toward tobacco control initiatives is scarce. This study assesses the attitudes and perceptions of public health students toward tobacco control initiatives. The study further assessed differences in attitudes and perceptions by country of origin.

Methods: A survey on attitudes and perceptions towards tobacco control policy was administered to a sample of 241 students classified as national and international, based on the country of origin. Most participants (78.8%) were originally from the U.S. Among WHO regions represented in the international group were Europe (35.3%) and Western Pacific (32.5%). Most international participants originated from countries with low income economies (56.9%).

Results: Preliminary data analysis revealed that most participants considered tobacco as a public health problem and priority in their respective countries of permanent residence. 'Policy and regulation' and 'health education' were ranked as the first and second most important measures for tobacco use prevention and control. Only 60.6% of national participants agreed that tobacco advertising should be legislated and restricted to certain locations and media. Many international participants (51.0%) concurred that tobacco advertising should be forbidden everywhere. Interestingly, 84.9% of the national participants and 50.0% of the international participants were not familiar at all with the "WHO Tobacco Day Initiative". However, there were significant differences among the two groups by age (0.016), gender ($p<0.001$) and occupations in the country of permanent residence ($p<0.001$). Further analysis will be necessary to determine the contribution of demographic variables to differences in attitudes and perceptions towards tobacco control.

Conclusions: The findings of this study characterize the attitudes and perceptions of tobacco control policy as generally positive. However, there are observed differences by various student characteristics. It is important that public health training in tobacco control policy and education is informed by these apparent differences.

Sponsor:

1711

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EPIDEMIOLOGIC ASSESSMENT OF TUBERCULOSIS IN CHILDREN. RESEARCH PROPOSAL

Purpose: The purpose of the present study is to investigate clinical and epidemiological aspects of tuberculosis in children to identify and assess risk factors, study the correlation between radiological and clinical findings, and estimate the influence of the country of origin of the source case for the childhood tuberculosis yield ratio between associate and contact investigations.

Methods: This research will be developed with data from a cross-sectional record review conducted at three sites of the Centers for Disease Control and Prevention TB Epidemiology Consortium Sites located in the States of California, New York, and Texas. Data includes childhood patients with latent and active tuberculosis. This research project has six different components: A comparison of active and latent TB will be conducted in regards to the presence or absence of selected variables to identify high risk groups and potential risk factors; the association of TB and history of BCG immunization will be explored by comparing TB cases and LTBI regarding their BCG immunization rates; a case-series analysis will be conducted to measure the frequency and percentage of each different chest x-ray radiological finding in asymptomatic TB cases; and the childhood TB yield rate and yield rate ratio of associate/contact investigations will be assessed and contrast among different country of origin of the source case.

Results: At least 90 cases of childhood tuberculosis and 400 children with latent tuberculosis will be included in this study. The results will identify high risk groups, risk factors, and clinical features in children with tuberculosis. In addition, the potential influence of the country of origin of the source case on the yield of tuberculosis detection will be studied by using the rate ratio of associate/contact investigations.

Conclusions: The information obtained from this study will be useful to learn more about risk factors that determine the likelihood to develop the disease in children five years of age and younger and will provide epidemiological and clinical profiles of TB patients five years of age and younger that can be used to identify high risk groups and develop TB control strategies to address diagnostic and epidemiologic problems of tuberculosis in this population that perhaps is the most vulnerable.

Sponsor: *CDC*

1712

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ASSOCIATION BETWEEN BREASTFEEDING AND ASTHMA

Purpose: Study the protective effect of breastfeeding and childhood asthma as controversy has surrounded this association.

Methods: The study included two different phases. The first phase included a systematic literature review of peer-reviewed articles and, the second phase, a cross-sectional study was conducted by using data from the National Health and Nutrition Survey (NHANES). Cases of asthma were compared with non-cases of asthma in regards to breastfeeding practices adjusting for potential confounders by using multiple logistic regression analysis.

Results: The literature review provided evidence of the protective effect of breastfeeding for children below six years of age, while in older children, adolescents and adults results seem controversial. A strong protective association was found for those who were breastfed in the cross-sectional study (OR = 0.693, 95%CI: 0.45, 0.91, p = 0.014). A dose-response relationship was also found as the number of months being breastfed increased, the prevalence of asthma decreased (p = 0.017).

Conclusions: Strong evidence confirms the protective association between breastfeeding and asthma in children below six years of age. Probably due to potential limitations in published literature, the protective effect of breastfeeding is not clear in older children, adolescents and adults. More longitudinal studies are needed to assess the potential benefits of breastfeeding in subjects older than five years of age.

Sponsor:

1713

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OSTEOPATHIC PHYSICIANS IN PRIMARY CARE, TEXAS, 2003

Purpose: To compare physician practice characteristics based on gender and medical education.

Methods: The Texas State Board of Medical Examiners complete electronic database for February 2002 wasused to identify Texas physicians in the public practice of medicine. Rates at which female and male DOs and MDs were engaged in specialty or primary care and at practice in a rural setting were compared. Statistical analysis using odds ratio and chi-square was used.

Results: Primary care osteopathic physicians are 1.4 times more likely to practice rural primary care than allopathic physicians. Taken relative to gender and medical degree type male osteopathic physicians are 2.3 times more likely than all other physicians to practice rural primary care and female osteopaths have the highest rate of primary care practice, with over 70% engaged in family or general practice, internal medicine, or pediatrics. Female osteopathic physicians have an odds ratio 4 times greater than other physicians to practice primary care and are 2.5 times more likely than female allopathic physicians to practice rural primary care.

Conclusions: Results from this study suggest that support of osteopathic medical education may result in increases in the primary care workforce as well as address the loss of rural practice locations.

Sponsor:

1800

Author: Eric Gonzales **Presentor:** Eric Gonzales
Department: PHARMACOLOGY & NEUROSCIENCE
Classification: GSBS Student
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THE 7' RESIDUE IN THE SECOND TRANSMEMBRANE DOMAIN OF LIGAND-GATED ION CHANNELS INFLUENCES RECEPTOR GATING KINETICS

Purpose: The cys-loop family of receptors is responsible for rapid neurotransmission in the central nervous system. Members of this family, which include the gamma-aminobutyric acid type A, glycine (GlyRs), nicotinic acetylcholine, and serotonin type-3 receptors (5-HT3Rs), share structural homology and are targets for numerous therapeutics. Each receptor is composed of five protein subunits, with four transmembrane domains each. Both the 5-HT3A and the glycine receptor can form functional homomers. Compared to other members of the LGIC superfamily, homomeric 5-HT3A receptors have significantly slower gating kinetics. The second transmembrane domain (TM2) lines the channel pore and these residues influence receptor gating. In the 5-HT3A receptor, mutation of the 7' leucine of the TM2 domain to threonine (L7'T) results in more rapid activation and deactivation rates and a decrease in ligand sensitivity (Das et al., Soc. Neursci. Abst., 2003). We have assessed the role of the TM2 7' position in channel kinetics of glycine receptors.

Methods: Wild type and mutant glycine alpha1 homomeric receptors were expressed transiently in HEK293T cells. Mutations (T7' to L, S, and A) were introduced by site-directed mutagenesis. We used the whole-cell patch clamp recording method to analyze each receptor and generate a glycine concentration response curve. Solutions containing glycine were applied using gravity flow via a Y-tube. Kinetic properties of the receptor were analyzed with a rapid solution exchange system (20-80% rise time ~ 14 ms) and computer analysis.

Results: The Gly alpha1(T7'L) mutation increased in sensitivity to glycine (EC50=15.9 uM from 29.2 uM), while the Gly alpha1(T7'S) and Gly alpha1(T7'A) mutations became less sensitive to glycine (EC50 values of 242.9 and 777.7 uM, respectively). Correlation analysis revealed no relationship between amino acid properties (volume, hydrophilicity, hydrophobicity, and hydropathy) and ligand sensitivity, while the activation kinetics at glycine EC50 concentrations were positively correlated with hydropathy at this position. Deactivation kinetics at 30xglycine EC50 concentrations are influenced by amino acid hydrophilicity.

Conclusions: These results demonstrate the involvement of the TM2 7' position of the cys-loop family of receptors in receptor gating. Future experiments will target the TM2 7' position and determine if this residue projects toward the channel pore or toward the protein background within the receptor.

Sponsor: *NIH ES 07904*

1802

Author: Zhenglan Chen **Presentor:** Zhenglan Chen
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THREONINE 112 PLAYS A KEY ROLE IN THE INHIBITORY MODULATION OF GLYCINE RECEPTORS BY COPPER

Purpose: Trace metals such as zinc (Zn2+) and copper (Cu2+) are stored in synaptic vesicles in the nerve terminals, and can be released into the synaptic cleft, reaching a high micromolar concentration. Both Zn2+ and Cu2+ have been shown to modulate voltage- and ligand- gated ion channels and thus modulate brain excitability. In particular, Zn2+ and Cu2+ modulate inhibitory GABA-A and glycine receptors. The site and mechanism for Cu2+ inhibitory modulation of glycine receptors is unknown. The goal of the present study was to identify amino acid residues involved in Cu2+-mediated inhibition of the glycine receptors.

Methods: Whole-cell glycine activated currents were recorded from human embryonic kidney (HEK) 293 cells expressing wild-type and mutant recombinant glycine receptors.

Results: Cu2+ reversibly inhibited glycine currents in a concentration-dependent manner in wild-type homomeric alpha1 and heteromeric glycine alpha1beta receptors. Extracellular histidine residues (H107/H109) on the a1 subunit have been reported to form the inhibitory binding sites for Zn2+ ions. Double histidine mutations for 107 and 109 did not affect Cu2+-induced inhibition. However, substitution of a hydroxylated residue threonine (T) 112 with a non-hydroxylated amino acid residue alanine (A) or phenylalanine (F) at the alpha1 subunit abolished inhibition of glycine-activated currents by Cu2+. Moreover, replacement of T112 with another hydroxylated residue (tyrosine) retained the receptor sensitivity to Cu2+.

Conclusions: Our data suggest that the hydroxyl group at T112 is critical for the action of Cu2+. The extracellular histidine residues are not involved in Cu2+ modulation, demonstrating the mechanism of Cu2+-mediated inhibition is distinct from that of Zn2+ .

Sponsor: *AHA TX 0160091Y*

1900

Author: Michael Gatch **Presentor:** Michael Gatch
Department: PHARMACOLOGY & NEUROSCIENCE
Classification: Faculty (Not for Competition)
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GABAERGIC MEDIATION OF THE DISCRIMINATIVE STIMULUS EFFECTS OF METHAMPHETAMINE

Purpose: Methamphetamine largely produces its effects through release of dopamine. However, GABA is known to modulate the dopamine system.

Methods: To test whether GABA modulation of dopamine is important in mediation of the discriminative stimulus effects of methamphetamine, the GABA compounds chlordiazepoxide (BZD receptor agonist) and PTZ (GABAA receptor noncompetitive antagonist) were tested in Sprague-Dawley rats trained to discriminate methamphetamine (1.0 mg/kg, i.p.) from saline. Ethanol was also tested as it produces many of its effects at GABAA receptors, and interaction of ethanol and methamphetamine is of interest as these compounds are often co-abused.

Results: Chlordiazepoxide (0.5 to 25 mg/kg, i.p.) dose-dependently blocked the discriminative stimulus effects of 1.0 mg/kg methamphetamine at doses which do not appreciably decrease response rates. Pentylenetetrazol (2.5 to 10 mg/kg) partially substituted in male rats (maximum 48% drug-appropriate responding at 10 mg/kg). Ethanol failed to substitute for or antagonize the discriminative stimulus effects of 1.0 mg/kg methamphetamine. When given in combination with a range of methamphetamine doses (0.1 to 1.0 mg/kg), ethanol produced a left-shift in the methamphetamine dose-effect curve.

Conclusions: These findings support the hypothesis that GABAA receptors modulate the discriminative stimulus effects of methamphetamine, and suggest a possible pharmacological mechanism for co-abuse of methamphetamine and ethanol.

Sponsor: *N01DA-2-8822*

1901

Author: Kelley Beck **Presentor:** Kelley Beck
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**all from University of North Texas Health Science Center, Ft Worth*

PSYCHOLOGICAL PROFILE PATTERNS AMONG THE MORBIDLY OBSESE: IMPLICATIONS FOR TREATMENT STRATEGIES

Purpose: This study investigated patterns of psychological functioning that may lead to improved prognostic indicators and post-operative interventions.

Methods: Participants included 75 extremely obese patients undergoing a presurgical evaluation at a Bariatric Surgery Clinic. Each participant completed the Millon Behavioral Medicine Diagnostic (MBMD) as part of the psychological test battery at the time of evaluation.

Results: A hierarchical agglomerative clustering technique was performed on all clinical scales of the MBMD using Ward's clustering technique with squared Euclidean distances. The mean age for the sample was 43.5 years (in a range of 23-68) and mean Body Mass Index (BMI) was 46.2 (in a range of 37-61). Three distinct clusters were identified. These clusters are primarily distinguished by differences and commonalities in the use of self-appraisal, relationships with others, affective functioning, and somatic focus as they relate to the chronic condition of obesity and its comorbid conditions.

Conclusions: Results indicate there are three broad classifications within a laparoscopic banding sample. Patients in cluster 1 present themselves as optimistic, confident, and relaxed. These patients present with no negative affective disturbance and are not overly-focused on somatic symptoms. However, acceptance by others is very important and they have no mechanism for handling rejection. These patients are most at risk for adjustment difficulties. Patients in cluster 2 view everything through a negative affective lens. Weight loss makes no difference to these patients because the weight loss has no effect on the negative lens through which they view themselves, the world, and the future. Patients in cluster 3 have primarily a somatic focus; their focus is on physical symptoms. Weight loss will have a significant positive impact on these patients. Overall, each of these clusters has different implications for treatment recommendations and prognostics.

Sponsor:

1902

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MEASURING DEPRESSION IN NURSING HOME RESIDENTS WITH THE MINIMUM DATA SET AND GERIATRIC DEPRESSION SCALE

Purpose: To examine the Minimum Data Set (MDS) and Geriatric Depression Scale (GDS) as measures of depression among nursing home residents.

Methods: Trained research nurses assessed residents using the MDS (version 2.0) and the GDS fifteen-item version. Demographic, psychiatric, and cognitive data were also obtained using the MDS. Level of depressive symptoms was operationalized as: (1) a simple sum of the MDS Depression items; (2) the MDS Depression Rating Scale (DRS); (3) the fifteen-item GDS; and (4) the five-item GDS. We compared missing data, floor effects, means, internal consistency reliability, scale score correlation, and ability to identify residents with conspicuous mental illness across cognitive impairment strata.

Results: The GDS and MDS Depression scales were statistically uncorrelated. Nevertheless, both MDS and GDS measures demonstrated internal consistency reliability. Means of MDS- and GDS-derived measures did not correspond across cognitive impairment groups. The MDS implied greater depression among the cognitively impaired, whereas the GDS implied a greater depression among the cognitively intact. The GDS was limited by missing data; the DRS by a larger floor effect. The DRS was a stronger correlate of conspicuous depression (diagnosis or antidepressant use), but only among the cognitively impaired.

Conclusions: The MDS Depression items and GDS identify different forms of depression. This may be due to differences in the manifest symptom content and/or the self-report nature of the GDS versus the observer-rated MDS. Our findings suggest that the GDS and the MDS are not interchangeable measures of depression.

Sponsor:

1903

Author: Michael Cutler **Presentor:** Michael Cutler
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CRANIO-SACRAL CV-4 CAN DECREASE SLEEP LATENCY IN HEALTHY HUMANS.

Purpose: The autonomic nervous system plays an important role in the homeostatic regulation of sleep/wake cycles. Recently, we demonstrated that the cranio-sacral still point is associated with decreased sympathetic nerve activity (JAOA 102(8): 437, 2002). The current study was designed to determine if cranio-sacral CV-4 alters sleep latency in humans.

Methods: Eleven subjects underwent a sleep latency test during each of three randomly ordered treatments (CV-4 trial, CV-4 sham trial, and control). During each trial ECG, BP, EEG, EOG, and EMG were recorded continuously. Sleep latency was determined using standard Multiple Sleep Latency Test protocol (Sleep, 9(4):519-24, 1986).

Results: Consistent with our hypothesis, a significant main effect for sleep latency between groups was observed ($p<0.001$). Specifically, sleep latency during the CV-4 trial was decreased when compared to both the CV-4 sham or control trials ($p<0.05$). Also, percent total sleep during each trial was different between groups ($p<0.01$). Percent total sleep during the CV-4 and CV-4 sham trials were different from control. However, there was no difference between percent total sleep during the CV-4 and CV-4 sham trials.

Conclusions: These data suggest that the cranio-sacral CV-4 technique can decrease sleep latency independent of touch.

Sponsor: *Funded in part by a research fellowship and grant from the American Osteopathic Association: F01-01 (MJC), 98-11-466 (MLS).*

1904

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HAPLOTYPE FREQUENCIES FOR PROMEGA'S POWERPLEX Y SYSTEM FOR SOUTHWESTERN HISPANIC AND ASIAN POPULATIONS

Purpose: To determine the mutational rate and haplotype frequencies of Promega's PowerPlex Y System for Asian and Southwestern Hispanic populations.

Methods: Obtain 200 father - son pair samples from the DNA Identity Lab at the University of North Texas Health Science Center from the Asian and Southwestern Hispanic populations. Use Promega's PowerPlex Y System to do PCR on the samples. Analyze them on a ABI 3100, and do statictical calculations on them to determine the mutational rate and haplotype frequenices.

Results: Only 192 father - son pair samples from the Southwestern Hispanic population was analyzed, along with 73 Asian father - son pair samples.

Conclusions: The mutation rate for Asians were higher then that of Southwestern Hispanics. The haplotype frequencies between the two showed some simularites and some differences between the two populations.

Sponsor:

1905

Author: Robert Kaman **Presentor:** Robert Kaman
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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, Minority K-12 Initiative for Teachers and Students (MKITS), The GO Project, and Bridges to the Doctoral Degree.

Methods: Each program is distinct, but offers summer research internships for college (SMART, McNAIR) experience in teaching science in K-12 (MKITS and SCORE), K-12 mentoring, tutoring and advising (Adopt-a-School, MKITS, SCORE, GO) support for graduate training (Bridges) and involvement of student organizations in a variety of activities (Adopt-a-School, MKITS, GO). Several partnerships with minority serving institutions have been developed, and a studentpipeline 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 Administration for its success. The National Association of Outreach Admissions Professionals named it the 1999 winnter of its Excellence in Minority Admissions award, and Minority Access, INC., has named the University of North Texas Health Science Center the role model institution.

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

Sponsor: *NIGMS, NHLBI, NSF,DOE, State of Texas Coordinating Board*

BIOMEDICAL SCIENCES ORAL PRESENTATIONS, SESSION I

2:00 PM	Leslie Don Roberts YING YANG-1 (YY1) DEFINES THE ACTIVATION THRESHOLD FOR SMOOTH MUSCLE MYOSIN HEAVY CHAIN PROMOTER ACTIVITY	Abstract# 333
2:20 PM	Nopporn Thangthaeng NOVEL TARGETING MECHANISM OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE IIB	Abstract# 501
2:40 PM	Swapnil Vaidya IN VIVO ROLE OF THE NATURAL KILLER CELL RECEPTOR 2B4 (CD244) IN TUMOR REJECTION	Abstract# 1001
3:00 PM	Nathalie McClung PROGESTERONE MODULATES INOSITOL 1,4,5-PHOSPHATE-MEDIATED CALCIUM SIGNALING IN BIPOLAR CELLS OF THE RODENT RETINA	Abstract# 1311
3:20 PM	BREAK	
3:40 PM	Shaohua Yang EFFECTS OF SEX STEROIDS ON STROKE	Abstract# 1308
4:00 PM	Renee Silvis HAPLOTYPE FREQUENCIES FOR PROMEGA'S POWERPLEX Y SYSTEM FOR SOUTHWESTERN HISPANIC AND ASIAN POPULATIONS	Abstract# 1904
4:20 PM	Eric Gonzales THE 7' RESIDUE IN THE SECOND TRANSMEMBRANE DOMAIN OF LIGAND-GATED ION CHANNELS INFLUENCES RECEPTOR GATING KINETICS	Abstract# 1800
4:40 PM	Athena Shephard CORRELATION BETWEEN MECHANICAL AND ENZYMATIC EVENTS IN SKELETAL MUSCLE FIBER	Abstract# 1603

BIOMEDICAL SCIENCES ORAL PRESENTATIONS, SESSION II

2:00 PM	**Yi Wen** CDK5 IS INVOLVED IN THE TRANSIENT CEREBRAL ISCHEMIA INDUCED ABERRANT NEURONAL CELL-CYCLE RE-ENTRY	**Abstract# 1313**
2:20 PM	**Jae-Kyung Lee** CHARACTERIZATION OF A NOVEL RECEPTOR CS1 IN HUMAN LYMPHOCYTES	**Abstract# 1002**
2:40 PM	**Krishna Gondi** BEHAVIORAL SENSITIZATION TO COCAINE AND METHAMPHETAMINE IN SWISS-WEBSTER MICE	**Abstract# 1303**
3:00 PM	**Sung-Yong Hwang** EFFECT OF PRESENILIN-1 ON INTRACELLULAR CALCIUM CHANNELS	**Abstract# 113**
3:20 PM	**BREAK**	
3:40 PM	**Anson Pierce** EFFECTS OF A NOVEL ALLELE FOR EXTRACELLULAR SUPEROXIDE DISMUTASE ON THE TISSUE PHENOTYPE IN MICE	**Abstract# 317**
4:00 PM	**Chang Su** TGFbeta1 DELAYS G2/M PHASE PROGRESSION VIA PKN SIGNALING IN VASCULAR SMOOTH MUSCLE CELLS	**Abstract# 326**
4:20 PM	**Everett Nixon** INTERACTIONS BETWEEN METABOTROPIC GLUTAMATE RECEPTOR TYPE 8 AND CALCIUM CHANNELS IS G-PROTEIN MEDIATED IN MOUSE ROD PHOTORECEPTORS	**Abstract# 1312**
4:40 PM	**Joel Ellis** MECHANISM of CaM KINASE IIdC SILENCING of MEF2-DEPENDENT GENE TRANSCRIPTION	**Abstract# 306**

PUBLIC HEALTH ORAL PRESENTATIONS

2:00 PM	**Bharat Mittal** A SURGICAL APPROACH TO CEREBRAL VASCULAR ACCIDENT (CVA)	**Abstract# 901**
2:20 PM	**Patrick Moonan** TOWARDS COMPUTATIONAL EPIDEMIOLOGY: APPLICATIONS IN EPIDEMIOLOGY AND COMPUTER SCIENCE	**Abstract# 1701**
2:40 PM	**Thaddeus Miller** A STANDARDIZED TOOL FOR ASSESSING TB SURVEILLANCE AND ACTION PERFORMANCE AND COST	**Abstract# 1702**
3:00 PM	**Vinay Parameswara** Y'ALL DOCTORS! I AIN'T FOR SURE I WANNA STAY HERE - A TEXAS PERSPECTIVE OF THE DISCHARGES AGAINST MEDICAL ADVICE	**Abstract# 1705**
3:20 PM	**BREAK**	
3:45 PM	**Ella Nkhoma** SPATIOTEMPORAL ANALYSIS OF ACCIDENTAL POISONING MORTALITY IN TEXAS (1980 - 2001): IMPLICATIONS FOR EMERGENCY PREPAREDNESS	**Abstract# 1709**
4:05 PM	**Marco Marruffo** EPIDEMIOLOGIC ASSESSMENT OF TUBERCULOSIS IN CHILDREN RESEARCH PROPOSAL	**Abstract# 1711**
4:25 PM	**Ann Trombley** ASSOCIATION BETWEEN BREASTFEEDING AND ASTHMA	**Abstract# 1712**

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