



UTHealth[™] |

Medical School

**The University of Texas
Health Science Center at Houston**



**2012 SUMMER RESEARCH PROGRAM
STUDENT ABSTRACTS**

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Contents

Preface	5
Acknowledgements	7
Lab Research Ownership	9
Index	
Medical Students	11
Undergraduate Students	12
International Medical Students	13
Abstracts – Medical Students	14
Abstracts – Undergraduates	87
Abstracts – International Medical Students	126

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Preface

The University of Texas Medical School at Houston (UTMSH) Summer Research Program provides intensive, hands-on laboratory research training for MS-1 medical students and undergraduate college students under the direct supervision of experienced faculty researchers and educators. These faculty members' enthusiasm for scientific discovery and commitment to teaching is vital for a successful training program. It is these dedicated scientists who organize the research projects to be conducted by the students.

The trainee's role in the laboratory is to participate to the fullest extent of her/his ability in the research project being performed. This involves carrying out the technical aspects of experimental analysis, interpreting data and summarizing results. The results are presented as an abstract and are written in the trainees' own words that convey an impressive degree of understanding of the complex projects in which they were involved.

To date, nearly 1,800 medical, college, and international medical students have gained research experience through the UTMSH Summer Research Program. Past trainees have advanced to pursue research careers in the biomedical sciences, as well as gain an appreciation of the relationship between basic and clinical research and clinical practice.

UTMSH student research training is supported by a grant from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Neurological Disorders and Stroke (NINDS), and/or by financial support from the Dean and the departments and faculty of the medical school.

Biomedical science education remains a vital and integral part of our nation's interests. The UTMSH Summer Research Program, and the dedication of our faculty and administration exemplify the institution's commitment to training and educating the future leaders in our biomedical scientific communities.



Gary C. Rosenfeld, Ph.D.

Director, Summer Research Program
Assistant Dean for Educational Programs

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Acknowledgements

This publication marks the completion of the twenty-fifth year of The University of Texas Medical School at Houston (UTMSH) Summer Research Program. The longevity and success of the program are rooted in the overwhelming support received from the deans, faculty, staff and students of the medical school.

Indicative of this support is the administrative assistance and financial support for the Program's college and medical students provided by UTMSH. Sincere appreciation is expressed to Dean Giuseppe Colasurdo M.D. and Patricia M. Butler, M.D., Associate Dean, Office of Educational Programs who continue to ensure the yearly success of the Summer Research Program.

Major financial assistance for medical students has also been provided through short term research grants by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; 5 T32 DK007676) and the National Institute for Neurological Disorders and Stroke (NINDS; 5 T35 NS064931).

Negotiated cooperative agreements with several international medical schools have been set up to offer tailored research programs at UTMSH for selected foreign medical students who interact fully with the other students in the Summer Research Program.

The success of the Summer Research Program depends primarily on the faculty who volunteer to mentor the trainees. These dedicated educators organize and guide the research projects that includes for each student data analysis, preparation of an abstract and public presentation of results. Our sincere appreciation to all faculty mentors.

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Lab Research Ownership

Publication and/or Disclosure

Each student participating in this program is required to read, agree to, and sign this disclosure form. The original signed copy is on file in the Summer Research Program office; the student and their faculty mentors are each furnished with a copy.

“In reference to the laboratory research you will perform this coming summer through The University of Texas Medical School at Houston’s Summer Research Program, you are required to comply with the standard restrictions regarding participation in the Summer Research Program:

“All of your laboratory research is *CONFIDENTIAL* and although your abstract will be available through our website, you cannot independently disclose or publish any research findings or data in any form (including at meetings or conferences) without the express prior written approval of The University of Texas Medical School at Houston. If you wish to submit your abstract to any third party, you must first contact your faculty mentor no less than three (3) weeks prior to any deadlines in order to obtain the necessary written approvals.

“Because your research was generated from ideas and funds that originated with your faculty mentor and The University of Texas Medical School at Houston, ownership of any data generated by you during the Summer Research Program belongs to The University of Texas Medical School at Houston or the Principle Investigator (PI).”

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Medical Students

Last Name	First Name	Page #	Last Name	First Name	Page #
Abraham	Jasson	15	Markey	Joseph	51
Alexander	Amy	16	Mayo	Sara Ayako	52
Atkinson	Joshua	18	McMullen	Ashley	53
Bell	Jessica	19	Mitchell	Kyle	55
Belton	Matthew	20	Moffitt	Olivia	56
Berry	Andrew	21	Murphy	Ryan	57
Chen	Angela	23	Oats	Sam	58
Clinton	Cameron	24	Parham	Chris	59
Cockerill	Katherine	25	Pieters	Thomas	60
Comer	Latoya	26	Polansky	Maxim	62
Darger	Bryan	27	Ray	Hunter	63
Doan	James	29	Reid	Matthew	64
Farhood	Zachary	30	Reusser	Nicole	65
Farley	Danny	31	Ricks	Christopher	66
Fuchs	Paul	32	Riney	Stephen	67
Gentry	Brandon	33	Robinson	Cory	68
Gianacakes	Eleni	34	Sahin	Hakan	69
Gonzales	Michael	35	Shah	Jaimin	70
Gover	Adam	36	Shah	Mital	72
Gregory	Abigale	37	Simmons	Roxanne	73
Hamme	Cristina	38	Staloch	Dustin	74
Harger	John	39	Sugg	Taylor	75
Heaps	Braiden	40	Tyson	William	76
Hnatow	Matthew	41	Vandelaar	Laura	77
Hoang	Ly	42	Wang	Kai	79
Jaber	Yasmeen	43	Ward	Norman	80
Johnson	Jayson	44	West	Christopher	81
Jooya	Neda	45	Wilcoxson	Brandon	82
Jordan	Roger	46	Wilkerson	Christopher	83
Kain	Joshua	47	Williams	Stephen	84
Knight	John	49	Zaibaq	Jenine	86
Kulkarni	Shradha	50			

Undergraduate Students

Last Name	First Name	Page #
Altemus	Samantha	88
Appelboom	Brittany	89
Awad	Andrew	90
Boktour	Michael	91
Brazelton	Alicia	93
Cheng	Yanshu	94
Cherches	Matthew	101
Cherry	Elyssa	95
Davis	Elizabeth	96
deGannes	Samantha	97
Gasic	Andrei	98
Gomez-Carlin	Rodrigo	99
Guo	Steven	100
Guthrie	Greer	101
Han	ByoungJun	102
Head	Jared	103
Heffler	Julie	104
Hopping	Grant	105
Hunter	Brittney	106
Jin	Jingxiao	107

Last Name	First Name	Page #
Kattchee	Lauren	108
Le	Minh Di	109
Lee	Stephen	96
Lin	Angela	110
Miller	Richard	111
Nemri	Katrina	122
Park	Jin Woo	112
Rodriguez	Louie	113
Rog	Colin	114
Rosenthal	Nathan	115
Sarker	Borna	116
Slater	Amy	117
Smith	Catherine	118
Tinger	Sophia	119
Tran	Matthew	120
Wawrose	Richard	121
Xiong	Youxin	122
Zhang	Xi	124
Zheng	Micha	125

International Medical Students

Last Name	First Name	Page #
Chien	Yung Shin	127
Hamaguchi	Toka	122
Kano	Shizuka	128
Li	Tong	131
Lin	Kuan Ting	132
Liu	Chin Hsuan	133
Liu	Hua Hsuan	134
Qu	Shen	135
Wang	Yu Ting	136
Xie	Yuan Hong	137
Yujiao	Wu	138
Zhou	Wei	139
Zhou	Yiwen	140

Medical Students

ABSTRACT

Surgical Care Improvement Project (SCIP)- based antibiotic prophylaxis compliance during pediatric appendectomies and associated Surgical Site Infections (SSIs)

JASSON ABRAHAM

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Class of 2015

Sponsored by: KuoJen Tsao, MD, Department of Pediatric Surgery

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, 5T35DK7676-20

Key Words: Surgical site infections, Prophylactic Antibiotic Adherence , Pediatric Appendectomies

Introduction: Surgical Site Infections (SSIs) are post-operative nosocomially acquired infections and account for 17% of all hospital acquired infections (2nd most) amongst hospital patients. The Surgical Care Improvement Project (SCIP) has identified proper administration of prophylactic antibiotics can reduce the risk of SSIs. To ensure compliance, these guidelines, including appropriate drug type, appropriate dosing, and appropriate timing of administration, were integrated in the pre-incisional surgical safety checklist (SSCL). The purpose of this study is to investigate the impact of SCIP compliance to antibiotic prophylaxis to decreased SSI infections in patients with pediatric appendicitis after integration in the SSCL.

Methods: A retrospective cohort study of pediatric appendectomies (< 18 years old) was conducted to analyze compliance with prophylactic antibiotics and SSIs. Antibiotics were measured as correct antibiotic type, dosage and timing in relation to incision time. Only patients that were that incorporated the SCIP in the SSCL were included. Univariate and multivariate analyses were used with p<0.05 considered significant

Results: 40 pediatric appendectomy procedures were observed and 4 patients (10%) developed SSIs. There were no differences in the risk of SSI in regards to compliance with SCIP despite significant overall adherence. (See table)

Conclusions: The results show that antibiotic compliance alone may not prevent SSIs. The major limitations of the study were the small sample size and other potential comorbidities potentially linked to SSIs. SSI prevention is more than just compliance to antibiotics; it is a multifactorial outcome which must be reduced through multiple interventions.

Variables	Outcomes		P -Values
	(+) SSI (N=4)	(-) SSI (N=36)	
Age (years)	10.87 (+/- 3.03)	11.01 (+/- 3.22)	p = 0.94
Weight (kg)	51.09 (+/- 31.56)	51.43 (+/- 23.79)	p = 0.98
Case Duration (min)	69.25 (+/- 15.17)	49.36 (+/- 29.89)	p = 0.20
Diagnosis			
Acute	1 (4.16%)	24 (95.84%)	p = 0.149
Gangrenous	1 (33.33%)	2 (66.67%)	
Perforated w/o Abscess	1 (16.67%)	5 (83.33%)	
Perforated w/ Abscess	1 (16.67%)	5 (83.33%)	
Overall SCIP Adherence	3 (9.09%)	30 (90.91%)	
Antibiotic Type Correct	4 (10%)	36 (90%)	p = 0.65
Dosage Correct	4 (11.11%)	32 (88.88%)	
Timing Correct	3 (8.57%)	32 (91.43%)	

ABSTRACT

Decorin Contributes to Ozone-Induced Airway Hyperresponsiveness and Pulmonary Inflammation

AMY L. ALEXANDER *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Richard A. Johnston, PhD, Department of Pediatrics

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: Decorin, Obesity, Airway Hyperresponsiveness

Obesity is a worldwide epidemic and is well-known to contribute to the development of cardiovascular disease, type 2 diabetes, and certain forms of cancer (Field *et al.*, *Arch Intern Med*, 2001). In addition, following exposure to the ozone (O₃), the major component of photochemical smog, airway responsiveness to methacholine is enhanced in obese as compared to lean subjects (Bennett *et al.*, *Inhal Toxicol*, 2007). At present, the molecular mechanisms mediating these responses have not been well-deciphered. Our laboratory has been using obese mice to further understand the mechanistic basis for this relationship. In fact, we have previously reported that genetically obese mice manifest increased airway responsiveness to methacholine and enhanced pulmonary inflammation following exposure to O₃ (Shore and Johnston, *Pharmacol Ther*, 2006). Obesity leads to chronic systemic inflammation, which is characterized by elevated systemic levels of hormones, cytokines, chemokines, and soluble cytokine receptors (De Heredia *et al.*, *Proc Nutr*, 2012). Decorin is an extracellular matrix glycoprotein, which has recently been shown to be elevated in obese humans and rodents (Bolton *et al.*, *Biologics*, 2012). Interestingly, decorin is also pro-inflammatory (Marchica *et al.*, *Am J Physiol Lung Cell Mol Physiol*, 2011). Thus, the purpose of this study was to determine the contribution of decorin to O₃-induced increases in airway responsiveness and pulmonary inflammation. To examine the effect of decorin in mediating pulmonary responses to O₃, we measured airway responsiveness to inhaled methacholine *via* the forced oscillation technique and then enumerated the number of macrophages and neutrophils in the bronchoalveolar lavage fluid (BALF) of wild-type, C57BL/6 mice and mice genetically deficient in decorin twenty-four hours following the cessation of a three hour exposure to filtered room air or O₃ (2 parts/million). There were no genotype-related differences in airway resistance (R_{aw}), the coefficient of lung tissue damping (G), or the coefficient of lung tissue elastance (H) following exposure to filtered room air. O₃ exposure increased responses to methacholine for indices of airway (R_{aw}) and parenchymal (G and H) oscillation mechanics. Furthermore, parenchymal responses to methacholine were decreased in decorin-deficient as compared to wild-type mice. O₃ exposure increased the numbers of BALF macrophages and neutrophils in wild-type and decorin-deficient mice, yet the number of macrophages and neutrophils were reduced in decorin-deficient as compared to wild-type mice following O₃ exposure. These results

demonstrate that decorin mediates increases in parenchymal responses to methacholine following O₃ exposure. In addition, our results demonstrate that decorin contributes to both macrophage and neutrophil emigration to the airspaces following O₃ exposure. These data suggest that obesity-induced increases in decorin may contribute to the enhanced airway responsiveness and pulmonary inflammation observed in obese mice, and thus, may serve as a therapeutic target to alleviate the toxic effects of inhaled O₃ in the obese population.

ABSTRACT

Bystander killing via retinal gap junctions mediates neural degeneration in retinitis pigmentosa model in Zebrafish

JOSHUA A. ATKINSON The University of Texas at Houston Medical School Class of 2015

Sponsored by: John O'Brien, PhD, Department of Ophthalmology and Visual Sciences

Supported by: National Institute of Neurological Disorders and Stroke, T35 NS 064931-3

Key Words: retinitis pigmentosa, Connexin35, rhodopsin

Retinitis Pigmentosa is progressive retinal degenerative disease characterized by night blindness and progressive tunnel vision until the patient is completely blind. It is caused by the death of rod photoreceptors due to mutations in rod-expressed genes and is commonly hereditary. The spread of photoreceptor cell death from rods to cones is still under debate. We suggest that gap junctions connecting photoreceptors are responsible for the transmission of apoptotic factors and we aim to show that by reducing gap junction coupling we will reduce the spread of apoptosis in the outer nuclear layer of the retina. The P23H rhodopsin mutation is one of the most commonly seen mutations in human retinitis pigmentosa. We injected Zebrafish embryos with a tol2 construct expressing a mouse rhodopsin cDNA carrying the P23H mutation and an epitope tag with a leading zebrafish opsin promoter. We also injected embryos with a construct containing a short hairpin RNA sequence designed to knockdown Connexin35 (Cx35) expression, our gap junction of interest. We cryostat sectioned and TUNEL stained sections from various ages from 4-17 dpf. Using confocal microscopy we saw expression of our mutant rhodopsin using Flagtag antibodies at all ages, as well as associated cell death in older embryos expressing the mutant opsin. After determining the appropriate embryonic age for measurement, we will label Cx35 to establish the efficacy of our short hairpin RNA to knockdown Cx35, and measure the levels of apoptosis between mutant rhodopsin/Cx35 knockdowns and solely mutant rhodopsin. This study will show the ability of apoptotic signals to pass through gap junctions, spreading cellular death. Measures to reduce gap junction coupling in events like retinitis pigmentosa or stroke could drastically reduce the level of neuronal death.

ABSTRACT

Effects of Extended Release Methylphenidate on Impulsivity in Children with an ASD and Significant ADHD Symptomatology

JESSICA E. BELL

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Class of 2015

Sponsored by: Deborah Pearson, PhD, Department of Psychiatry and Behavioral Sciences

Supported by: The Bernard Saltzberg Summer Research Fellowship, Department of Psychiatry and Behavioral Sciences

Key Words: Autism, ADHD, methylphenidate

A substantial proportion of children with Autism Spectrum Disorders (ASD) (14-48%) are reported to have symptoms of inattention, hyperactivity, and impulsivity that are severe enough to warrant a DSM-IV diagnosis of Attention Deficit Hyperactivity Disorder (ADHD) – although a formal diagnosis is not allowed using current DSM-IV-TR guidelines. The purpose of this study was to determine if treatment with extended release methylphenidate (MPH) was associated with improvements in impulsivity, and to determine if the MPH dose-response curve was linear (i.e., higher MPH doses associated with consistent improvements in impulsivity), or curvilinear (e.g., MPH associated with an initial improvement, followed by lesser improvements and/or possible deterioration at higher doses). During the trial, each child received one week of each of the four doses (placebo, low, medium, and high) of a treatment regimen combining extended release MPH in the morning with immediate release MPH in the afternoon. At the end of each week, the children performed a delay of gratification task, which measures the ability to suppress or delay impulsive behavioral responses. Performance was measured by the number of correct responses and the efficiency ratio (number of correct responses/total number of responses). The correct responses increased in a linear fashion with successively higher doses of MPH while there was no significant change in efficiency ratio, which suggests that higher MPH doses on average are associated with successive improvements in the dose range studied. These findings indicate that impulsivity, as measured by a delay of gratification task, improved with MPH treatment in children with ASD who have clinically significant symptoms of ADHD.

ABSTRACT

Analysis of epidermal nerve fiber density utilizing skin biopsies in pediatric patients with dysautonomia

MATTHEW D. BELTON *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Ian J. Butler, MD, Mohammed Numan, MD, Department of Pediatrics

Supported by: Ian J. Butler, MD, Department of Pediatrics; University of Texas at Houston Medical School – Office of the Dean

Key Words: Dysautonomia, small fiber neuropathy, dysesthesia, paresthesia, peripheral ischemia

Patients diagnosed with dysautonomia often complain of dysesthesias, paresthesias, and numbness of the extremities in addition to headaches, syncope, and dizziness. Dysesthesia can be clinically categorized as a small fiber neuropathy affecting afferent sensory nerves while often sparing neuromuscular conduction. Our study examined the density pattern of small afferent A- δ myelinated and unmyelinated C fibers from punch biopsies (distal/proximal lower leg, distal thigh, and distal forearm) obtained from pediatric patients who complain of dysesthesias, are positive for neurocardiogenic syncope using standard tilt table testing, and have had a normal EMG and nerve conduction studies. Intraepidermal sections stained with monoclonal antibodies specific to ubiquitin hydroxylase, PGP-5, showed both non-length dependent and length-dependent neuronal degeneration patterns based on adult control standards. All samples showed axonal swellings. We propose these findings are consistent with a systemic neural crest migration deficiency, affecting the autonomic nervous system and sensory pathways as well as other neural crest derivatives. During times of postural orthostatic intolerance, we propose that ischemic hypoperfusion of tissues may further damage small nerve fibers resulting in dysesthesias. Additional patient recruitment and pediatric control standards should provide insight into the severity of the neuropathy as well as confirm the length dependent relationship of the neuropathy.

ABSTRACT

Retrospective analysis of damage control surgery versus single stage procedures in trauma patients undergoing exploratory laparotomy

ANDREW R. BERRY *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Bryan A. Cotton, MD, MPH, Department of Surgery

Supported by: Bryan A. Cotton, MD, MPH, Department of Surgery, Center for Translational Injury Research (CeTIR); University of Texas at Houston Medical School - Office of the Dean

Key Words:

Background: The use of damage control surgery (DCS) in the management of trauma patients has led to numerous lives saved. Initially born of the necessity for rapid intervention in unstable patients with intra-abdominal injuries, DCS has evolved into the critical mediator between mechanical hemostasis and the medical management of the coagulopathy of trauma. In damage control laparotomy (DCL), an abbreviated surgery is performed to minimize physiologic perturbations, specifically the lethal triad of metabolic acidosis, hypothermia and coagulopathy. While it has been shown that DCL has a direct correlation with improved survivability of blunt or penetrating abdominal trauma, little has been done to show the systemic consequences of DCL in trauma patients.

Study Population: Patients meeting the inclusion criteria of (1) having undergone exploratory laparotomy for trauma, (2) evaluated/admitted between 01/01/04 and 7/25/12, (3) more than 18 years of age. We excluded patients who were (1) less than 18 years of age, (2) pregnant, (3) prisoners, (4) readmissions or follow-ups. Data from this center is being retrieved from electronic medical records (EMR), hospital charts, and the Trauma Registry of the American Academy of Surgeons. All patient identifiable information is secured in accordance with HIPAA rules and regulations and will be de-identified prior to final analysis.

Data collection:

- Demographics - age, gender, mechanism of injury, past medical history
- Injury characteristics - abbreviated injury severity scores, intra-abdominal injuries, other injuries related to the trauma, cause of bleeding.
- Complications such as but not limited to open abdominal wound complications (which includes how many days abdomen open, how closed and how many times abdomen was packed), orthopedic hardware infections, acute respiratory distress syndrome (ARDS), and deep vein thrombosis (DVT)
- Hospital course/outcomes - number of exploratory laparotomies, fluids received, post-operative complications, infections, vital signs, diagnostic results, number of ventilator days, ICU days, hospital days, discharge status (i.e. home, skilled nursing facility, long term acute care, etc.).

Objective: to assess the incidence rate of post-traumatic injury complications in patients undergoing damage control versus traditional exploratory laparotomy for trauma.

Hypothesis: Patients who undergo DCL will have, overall, a lower incidence of complications compared to patients undergoing single stage laparotomies for trauma.

Results: as of 8/3/2012, data collection is ongoing and therefore no results are available at this time.

ABSTRACT

Susceptibility to Out-of-Hospital Deaths in Bicuspid Aortic Valve Disease

ANGELA B. CHEN

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Class of 2015

Sponsored by: Siddharth Prakash, MD, PhD, Divisions of Cardiology and Medical Genetics

Supported by: Volunteer

Key Words: BAV, TAAD, aortic disease

Bicuspid aortic valve disease (BAV) affects about 0.5-2% of the population and is the most common congenital cardiac malformation. BAV results from incomplete separation of the endocardial cushions, giving rise to a malformed valve with partially or completely fused derivative cusps. While BAV is often characterized as an isolated defect, it may also involve additional cardiac and vascular manifestations. Patients have a markedly increased risk for thoracic aortic aneurysms and acute aortic dissections (TAAD), and most patients eventually develop aortic stenosis or regurgitation. Despite its importance, the natural history of BAV disease remains poorly understood, and the long-term prognosis of most patients, including the causes of death, is not known. BAV is heritable and associated with a number of genetic diseases and congenital cardiac lesions. The goal of our study is to investigate the causes for out-of-hospital deaths due to BAV disease in a population that has not been previously studied. BAV disease may be caused by complications of other congenital lesions such as undiagnosed genetic syndromes or congenital cardiac defects. This summer, I pioneered this study by establishing a close collaboration with the assistant medical examiner, which facilitated my access to the Harris County Institute of Forensic Sciences (IFS). Working with Dr. Kathryn Haden-Pinneri at IFS, I collected blood samples, photographs, autopsy reports and death investigation reports from decedents at the morgue with BAV and/or thoracic aortic disease. I extracted clinical information from these records and from the decedents' medical records when available and collated these data using descriptive and statistical analyses. I also performed the first systematic analysis of BAV morphology in autopsy specimens using current methods. Our long-term goal is to identify correlations between BAV phenotypes and outcomes with other congenital disorders or genetic mutations. DNA will be extracted from whole blood for sequence analysis and genome-wide association studies.

ABSTRACT

Moyamoya Disease and Family History of Premature Vascular Disease

CAMERON K. CLINTON *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Dianna M. Milewicz, MD, PhD, Internal Medicine – Medical Genetics

Supported by: Dianna M. Milewicz, MD, PhD, Internal Medicine – Medical Genetics;
University of Texas at Houston Medical School – Office of the Dean

Key Words: Moyamoya disease, vascular disease, stroke

Moyamoya disease (MMD) is a cerebrovascular condition that is marked by a progressive bilateral stenosis of the intracranial internal carotid arteries, leading to a compensatory development of collateral vasculature by small vessels. Familial MMD has been described in 15% of patients and demonstrates an autosomal dominant inheritance pattern with incomplete penetrance. We believe that the alterations in inherited genes are likely causing a spectrum of vascular disease in individuals who carry these alterations, with MMD at the severe end of the vascular disease spectrum. Our hypothesis is that this will manifest as families of patients with MMD having a greater burden of premature vascular disease defined as men ≤ 55 and women ≤ 60 . Individuals with MMD were enrolled in the Genetic Basis of Vascular Disease (GBVD) study at UTHSC-H and control pedigrees were collected in the Pediatrics and General Medicine clinics at UTHSC-H from patients without vascular disease. These two patient populations were matched for age, race, and sex. Data on their family history of premature vascular disease including aneurysm/dissection, stroke, myocardial infarction, stenosis, Coronary Artery Disease, sudden death, and MMD were abstracted from the family histories and an odds ratio analysis was performed. Our preliminary data analysis indicated a strong association between MMD and a family history of premature vascular disease, with odds ratios of 7.14, 6.99, and 6.98 for First degree, second degree and all relatives, respectively, all with p-values $< .001$ which provides some evidence for our hypothesis that the genes underlying moyamoya are possibly also responsible for other forms of premature vascular disease.

ABSTRACT

Impact of resistin deficiency on pulmonary responses to acute ozone exposure in mice

KATHERINE J. COCKERILL *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Richard A Johnston, PhD, Department of Pediatrics

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, 5T35DK7676-20

Key Words: Resistin, Ozone, Obesity, Pulmonary inflammation

Obese humans exhibit enhanced decrements in pulmonary function induced by exposure to ozone (O₃), a common environmental pollutant, when compared to those of normal weight (Bennet, *WD Inhal Toxicol* 2007 and Alexeeff, *SE Chest* 2007). However, the mechanistic basis for this relationship is not known. Our laboratory has been using obese mice to better understand this relationship, and we have previously reported that obese mice exhibit increased airway responsiveness to methacholine and enhanced pulmonary vascular hyperpermeability and pulmonary inflammation following exposure to O₃ (Johnston, *RA J Appl Physiol* 2008). Obesity increases the systemic levels of the hormone and pro-inflammatory cytokine, resistin, in humans. Therefore, the purpose of this study was to investigate the contribution of resistin to the development of O₃-induced airway hyperresponsiveness (AHR), pulmonary vascular hyperpermeability, and pulmonary inflammation. To that end, we exposed wild-type C57BL/6 and mice genetically deficient in resistin (Retn^{-/-}) to O₃ [2 parts/million (ppm)] or filtered room air for three hours. Twenty-four hours following the cessation of exposure, we measured airway responsiveness to methacholine using the forced oscillation technique and pulmonary vascular permeability and pulmonary inflammation using biochemical and histological analyses of tissue extracts. O₃ exposure increased the levels of resistin in the bronchoalveolar lavage fluid (BALF) in wild-type mice. Following exposure to air, there was no difference in airway responsiveness to methacholine between wild-type and Retn^{-/-} mice. O₃ exposure increased airway responsiveness to methacholine in both wild-type and Retn^{-/-} mice. However, there were no genotype-related differences in responsiveness to methacholine following O₃ exposure. BALF protein (Bhalla, *J Toxicol Environ Health B Crit Rev* 1999), a sensitive indicator of pulmonary vascular permeability, was not different between wild-type and Retn^{-/-} mice following air exposure. O₃ exposure increased the levels of BALF protein, but again, no genotype-related differences in BALF protein existed following O₃ exposure. The number of macrophages and neutrophils were also elevated following O₃ exposure in both genotypes, but no statistically significant differences existed in the number of macrophages and neutrophils between wild-type and Retn^{-/-} mice twenty-four hours following the cessation of O₃ exposure. These results suggest that resistin does not mediate O₃-induced AHR, pulmonary vascular hyperpermeability, and pulmonary inflammation. Furthermore, these results also suggest that elevated levels of resistin are unlikely to contribute to the enhanced pulmonary responses to O₃ observed in obesity.

ABSTRACT

Persistent Intestinal Inflammation in the Pathogenesis of Irritable Bowel Syndrome

LATOYA COMER

The University of Texas at Houston Medical School

Class of 2015

Sponsored by: Herbert DuPont, MD; Charles Darkoh, PhD, The University of Texas-Houston, School of Public Health

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, 5T35DK7676-20

Key Words: IBS, PI-IBS, ID-IBS

Introduction: Irritable bowel syndrome (IBS) results in a chronic, relapsing, and idiopathic inflammation of the gastrointestinal tract as a consequence of aberrant activation of immune response. Annually, IBS affects about 1.4 million and 2.2 million in the U.S. and Europe, respectively. IBS can be classified as either idiopathic (ID-IBS) with unknown etiology or post-infectious (PI-IBS), which develops after a bout of acute diarrhea or gastroenteritis. Little is known about the immunopathogenesis of these two forms of IBS. We sought to investigate the expression patterns of cytokines and chemokines present in the serum of IBS patients based on the hypothesis that the immunologic presentation of ID-IBS and PI-IBS is similar despite their different etiological origins.

Methods: Sera from 40 healthy controls and 60 IBS-patients (16 PI-IBS and 44 ID-IBS) were evaluated for the presence of cytokines, chemokines, and acute phase proteins using the Proteome Profiler Human Cytokine Array Panel A kit (R&D Systems, Minneapolis, MN) and ELISA.

Results: The concentration of the chemokine CXCL16 was significantly higher in ID-IBS ($p < 0.0001$) and PI-IBS ($p < 0.0001$) compared to the controls. However, no significant difference was found between ID-IBS and PI-IBS ($p = 0.5605$). Expression levels of pro-inflammatory cytokines (IL-1 β , IFN- γ , and TNF- α) were similar in ID-IBS and PI-IBS but significantly higher ($p < 0.001$) compared to the controls. Levels of anti-inflammatory cytokines (IL-10, IL-4, and IL-13) were variable but IL-10 was significantly higher ($p < 0.001$) in the controls than IBS.

Conclusions: Inflammation is a major hallmark of IBS. IBS patients express high levels of pro-inflammatory cytokines and chemokines and low levels of anti-inflammatory cytokines. The expression patterns of cytokines in the sera of ID-IBS patients were similar to that of PI-IBS, indicating a similar immunological response and clinical phenotype. An increase in IFN- γ and a decrease in IL-10 suggest that IBS may affect TH1/Th2 balance

ABSTRACT

Safety and Efficacy of tPA administration in Ischemic Stroke Patients Requiring Aggressive Blood Pressure-Lowering Treatment

BRYAN F. DARGER

The University of Texas at Houston Medical School

Class of 2015

Sponsored by: Pratik Doshi, MD, Department of Emergency Medicine

Supported by: National Institute of Neurological Disorders and Stroke, 5 T35 NS 64931-3

Key Words: Acute ischemic stroke, tPA, hypertension, labetalol, nicardipine

Introduction: Patients suffering acute ischemic stroke (AIS) often present to emergency departments with elevated blood pressure. Current guidelines and protocols in place at many hospitals recommend against tPA administration in patients with systolic blood pressures exceeding 185/110 mmHg after “standard” blood pressure lowering treatment as defined as up to 40 mg of IV Labetolol, or those requiring aggressive methods (IV medication) to achieve such levels. This precludes a large percentage of patients from receiving the only known therapy for acute ischemic stroke, even when they present within the limited therapeutic window of 4.5 hours from symptom onset. In this study, we sought to assess the safety and efficacy of tPA administration in patients presenting to the emergency department within 4.5 hours of symptom onset who require “aggressive” blood pressure management prior to tPA administration.

Methods: We evaluated the outcomes of patients who presented to the Memorial Hermann ED with elevated blood pressure who were given standard therapy with Labetalol HCl or aggressive therapy with Nicardipine IV prior to treatment with tPA and compared them to a control group of patients who did not require any blood pressure management prior to tPA administration. We performed a retrospective chart review of patients presenting to the ED from 2004-2011 who were treated with tPA for AIS.

Results: A total of 427 patient records were included in the analysis. Of these, 273 required no blood pressure management prior to tPA administration, 65 required “standard” blood pressure management, and 89 required aggressive blood pressure management prior to tPA administration. Main outcome measures were all grades of hemorrhagic transformation, neurologic deterioration, good outcomes (modified Rankin score of 0-2), and in-hospital deaths. Patients requiring BP control appeared to be older, had higher baseline NIHSS, initial glucose concentration, history of hypertension, and were more likely to be women. Of these parameters only female sex and history of hypertension reached significance. The rates of hemorrhagic transformation and in-hospital deaths appeared to be increased in the group that required any blood pressure control when compared to patients that did not require blood pressure control (6% vs. 3%, $p=0.1770$ and 11% vs. 8%, $p=0.86$) and the rate of good outcome lower in the group requiring blood pressure control (29% vs. 36%, $p=0.41$). However, none of these findings were statistically significant in multivariate analysis when adjusted for age, glucose and baseline NIHSS. When patients requiring “standard” vs. “aggressive” blood pressure control were

compared, the group requiring “aggressive” blood pressure control appeared to be older, were more likely to be women, and had higher baseline NIHSS, initial glucose concentration, and initial blood pressure. Only the baseline NIHSS and initial blood pressure reached statistical significance. The rates of any hemorrhagic transformation and in hospital deaths were higher in the “aggressive” blood pressure group (8% vs. 3%, $p=0.26$ and 14% vs. 6%, $p=0.54$) and rates of good outcome were lower in the “aggressive” blood pressure group (21% vs. 39%, $p=0.14$). After multivariate analysis adjusting for age, glucose and baseline NIHSS, only the lower rates of good outcome had a trend towards significance. There was also a trend towards significance for an increased length of stay for the group requiring any blood pressure management as compared to the group not requiring any blood pressure management prior to tPA. Additionally, there was no statistically significant difference in door to tPA time for the group requiring blood pressure management vs. the group not requiring blood pressure management (71.5 min vs. 69 min) and this was also the case when comparing the group requiring “standard” vs. “aggressive” blood pressure management (68 min vs. 76 min).

Conclusions: Administration of tPA in patients presenting with AIS requiring blood pressure management, even “aggressive” measures, appears to be safe and does not seem to be associated with worse outcomes. With integrated efforts, the need for blood pressure management does not need to be associated with delays in administration of tPA. Thus, the need for blood pressure management prior to tPA administration should not be an exclusion criteria for thrombolysis in patients presenting with acute ischemic stroke.

ABSTRACT

Decreased glycolysis diminishes contractile function of the insulin-resistant rat heart following ischemia, even though glucose oxidation is unimpaired

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Sponsored by: Heinrich Taegtmeier, MD, DPhil, Department of Internal Medicine

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: Heart, insulin resistance, ischemia, ER stress

The lab recently reported that insulin resistance (IR) preserves metabolic and contractile efficiency in stressed rat hearts (FASEB Journal 2012; published ahead of print May 18). However, in diabetic patients with a heart attack, morbidity and mortality are significantly increased. We therefore tested the hypothesis that alterations in myocardial glucose metabolism are linked to contractile dysfunction in ischemic, reperfused hearts from IR animals. To induce myocardial IR, male Sprague Dawley rats were fed a high-sucrose diet (67% of total kilocalories) for 8 weeks. Hearts were then **perfused *ex vivo* in the working mode** with glucose (25mM), oleate (0.8mM), and insulin (5ng/ml). After 15 min total global ischemia, hearts were reperfused for 30 min. Rates of glucose uptake, lactate release (glycolytic flux), and glucose oxidation were measured and expressed in nmol/min/gram dry weight. At the end of the protocol, **hearts were freeze-clamped and proteins were extracted** for immunoblot analyses. At baseline, myocardial insulin resistance was associated with a 17% decrease in the rate of glucose uptake (5006±298 vs. 6014±355; p<0.05) compared to chow-fed controls. Rates of lactate release were similarly decreased (9118±479 vs. 11013±733; p<0.05), while rates of glucose oxidation and cardiac power did not differ. During reperfusion, myocardial rates of glucose uptake were similarly decreased for sucrose (3246±217; p<0.001) and for chow-fed (3478±266; p<0.001) rats. Glycolytic flux was depressed in the insulin-resistant heart (9035±330 vs. 10406±510; p<0.05), while rates of glucose oxidation did not change. **Reduced glycolytic flux at reperfusion was associated with a marked impairment of cardiac power** compared with controls (28.0±1.1 vs. 36.1±1.4 mWatts/gram dry weight; p<0.001). Impaired contractile function of the insulin-resistant heart was not the result of increased cardiac cell death. However, increased phosphorylation of eukaryotic initiation factor 2 (eIF2α) and inositol-requiring enzyme 1 (IRE-1α), as well as increased splicing of the mRNA encoding X-box binding protein 1 (XBP1), indicated a **strong activation of the endoplasmic reticulum (ER) stress response**; this suggests that reduced glycolytic flux impairs calcium homeostasis. **In conclusion**, I have shown for the first time that decreased rates of glycolytic flux, rather than impaired rates of glucose oxidation, are associated with poor recovery of contractile function for the insulin-resistant heart, most likely as a result of ER stress and impaired calcium cycling.

ABSTRACT

Evaluating the Adverse Effects of Compounded Fluticasone Propionate Irrigations in Treating Chronic Rhinosinusitis

ZACHARY FARHOOD *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Amber Luong, MD, PhD, Department of Otorhinolaryngology – Head and Neck Surgery

Supported by: Amber Luong, MD, PhD, Department of Otorhinolaryngology – Head and Neck Surgery; University of Texas at Houston Medical School – Office of the Dean

Key Words: Fluticasone Propionate, CRS, ESS, FESS

Background: Fluticasone propionate is a corticosteroid commonly used as a topical agent instilled into the nasal cavity for the treatment of chronic rhinosinusitis (CRS). However, its bioavailability and therefore its potential adverse effects have not been previously evaluated when used to irrigate the paranasal sinuses of post-endoscopic sinus surgery (ESS) patients at high doses.

Methods: To evaluate the safety of fluticasone in this population when used at 3 mg BID, we measured salivary cortisol, intraocular pressure, and the development of clinical cataracts in patients before and after 6 weeks treatment with intranasal irrigations of high dose fluticasone during post ESS care as biomarkers for potential side effects. Nasal endoscopy and standardized clinical outcome questionnaires were performed to assess the drug's effectiveness at treating CRS. Up to 40 subjects will be enrolled with a minimum of 20 completing treatment. The results will be preliminarily analyzed using Wilcoxon rank sum tests.

Results: The study is in progress; 7 subjects have completed the study and an additional 13 subjects are enrolled. Results for the full study will be analyzed when at least 20 subjects have completed the follow up. Preliminary results on 7 subjects demonstrate no significant change in any of the three aforementioned variables when analyzed with the Wilcoxon rank sum test ($p < 0.5$).

Conclusions: We demonstrated no significant adverse safety events or biomarkers of potential risk for safety concerns in our analysis of the first 7 patients in a short term observational study of the safety of high dose fluticasone irrigation after ESS. We observed no clinically or statistically significant changes from baseline in salivary cortisol levels, intraocular eye pressure, and the presence of cataracts. Efficacy of the irrigations remains to be evaluated.

ABSTRACT

An Evaluation of Rapid Thromboelastography (rTEG) Velocity Curves in Predicting Large Volume Transfusions and Mortality

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Sponsored by: Bryan Cotton, MD, Center for Translational Injury Research

Supported by: Bryan Cotton, MD, Center for Translational Injury Research; University of Texas at Houston Medical School – Office of the Dean

Key Words: trauma, hemorrhage, massive transfusion, rapid admission thromboelastography, retrospective review, velocity curve

The purpose of this study was to investigate if often overlooked velocity curve parameters are more predictive of hemorrhage and early death than standard admission rapid thromboelastography curves. Admission rapid thromboelastography (r-TEG) provides real time information on the clotting status of severely injured patients. Standard r-TEG values such as, r-time, alpha angle, and max amplitude have been shown to predict transfusion rates, mortality, and pulmonary embolus. However, the parametric velocity curve values that generate the standard curve have been poorly investigated. Animal and healthy volunteer data has suggested that the rate and amount of thrombin generation and lysis may be predictive of the risk of hemorrhage, but little clinical research has been conducted. This was an 18-month retrospective review, including patients arriving with the highest level of trauma activation within 6 hours of injury. The data suggests that admission fibrinolysis velocity curves are stronger predictors of early mortality than standard r-TEG values.

ABSTRACT

Purposeful Variable Selection and Stratification to Impute Missing FAST Data in Trauma Research

PAUL FUCHS

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Class of 2015

Sponsored by: Deborah del Junco, PhD, Bryan Cotton, MD, Center for Translational Injury Research and Department of Surgery
Erin Fox, PhD, Center for Clinical and Translational Sciences

Supported by: Deborah del Junco, PhD, Center for Translational Injury Research

Key Words: FAST exam, impute, missing completely at random, missing at random

The Focused Assessment with Sonography for Trauma (FAST) exam is an important variable in many retrospective trauma studies. The purpose of this study was to devise an imputation method to overcome high missing data rates for the FAST exam. Due to variability in patients' injuries and trauma care, these data are unlikely to be missing completely at random (MCAR), raising concern for validity when analyses exclude patients with missing values. Imputation was conducted under a less restrictive, more plausible missing at random (MAR) assumption. Patients with missing FAST exams had available data on alternate, clinically relevant elements that were strongly associated with FAST results in complete cases, especially when considered jointly. Subjects with missing data (32.7%) were divided into eight mutually exclusive groups based on selected variables that both described the injury and were associated with missing FAST data. Additional variables were selected within each group to classify missing FAST data as positive or negative, further reducing bias. Correct FAST exam classification using these variables for patients with non-missing FAST values was determined. Severe head/neck injury (2.04), severe extremity injury (4.03), severe abdominal injury (1.94), no injury (1.94), other abdominal injury (0.47), other head/neck injury (0.57) and other extremity injury (0.45) groups had significant odds ratios for missing data; the other (0.84) group odds ratio was not significant. All 407 missing FAST values were imputed, with 109 classified as positive. Correct classification of non-missing FAST results using the alternate variables was 87.2%. Imputation for missing FAST exams based on interactions among selected variables may be a useful adjunct to sensitivity analysis in the evaluation of imputation strategies under different missing data mechanisms. The imputation method designed in this study has the potential for widespread application in clinical and translational research. Future studies should focus on validating this method.

ABSTRACT

Acivicin Inhibits Proliferation in Multiple Myeloma

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Supported by: Volunteer

Key Words: Multiple Myeloma, Acivicin, GMP Synthetase

The principal objective of the proposed study is to determine the anti-myeloma activity of the glutamine analog antimetabolite acivicin against pre-clinical models of multiple myeloma. Human myeloma cell lines (MM1, U266) were cultured for 72h with 5 μ M acivicin. Exogenous adenosine monophosphate and guanosine monophosphate were added as negative and positive controls. The antimyeloma activity was evaluated using the WST-1 cell proliferation assay. All treatments exhibited decreased proliferation except for those treated with exogenous GMP. The present study supports acivicin as a promising antimyeloma agent.

ELECTRONIC DRAFT: Trypan Blue analysis of cell viability and Western Blot analysis of GMP Synthetase Expression to be included.

ABSTRACT

Efficacy of a novel tolerogenic protein expressed by a Salmonella vector for diminishing anti-FVIII response in a mouse model of Hemophilia A

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Sponsored by: Keri C. Smith, PhD, Department of Pathology and Laboratory Medicine

Supported by: Keri C. Smith, PhD, Department of Pathology and Laboratory Medicine;
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Key Words: Hemophilia A, FVIII, tolerogen

Patients who have genetically acquired Hemophilia A have a mutated form of FVIII, which plays a key role in the clotting cascade. Exogenous recombinant FVIII is administered to these patients to prevent or stop bleeding. Approximately 25% of Hemophilia A patients develop an antibody mediated response against the administered FVIII, which inhibits activity of subsequent therapeutic FVIII doses. Hence, there is significant interest in development of methods to modulate the immune response to prevent development of inhibitor antibody. A novel tolerogen, CFA/I fimbriae, has previously been demonstrated to ablate autoimmune disease symptoms. Preliminary studies in the Smith laboratory using purified CFA/I fimbriae to immunize mice indicated that CFA/I fimbriae could reduce inhibitor titer concurrent with an increase in regulatory T cells and a decrease in the pro-inflammatory cytokine IL-17. However, the effects appeared to be short-lived. Since CFA/I fimbriae can be stably expressed by attenuated *Salmonella typhumurium*, we asked if inoculation of mice with this strain concurrent with a FVIII injection would lead to long term CFA expression and subsequent reduction in immune response. In our experiments, we compared the effects of *S. typhumirium* stably expressing CFA/I fimbriae versus and control of the same bacteria with an empty vector on the production of anti-FVIII and inflammatory cytokines. We also compared oral versus nasal route of inoculation. Unfortunately, repeated inoculation with the bacteria was associated with significant mortality (approx. 22%). Regardless of route of administration, neither control or tolerogenic vector reduced anti-F8 antibody titer. In contrast to the previous lab results, we did not observe reduction in IL-17 or increase in regulatory T cells following bacterial inoculation. However, when further assays were conducted, we discovered a lack of detectable antibody against the CFA/I fimbriae tolerogen suggesting that the expression of the protein might be compromised. Moreover, the mice failed to secrete detectable inflammatory cytokines in response to stimulation with FVIII. Therefore, we cannot discount the possibility that mucosal infection with the salmonella is somehow redirecting the immune response to FVIII. The inability of Salmonella expressing CFA/I fimbriae to reduce anti-FVIII titer as well as its unwanted side effects makes it unlikely to be a reasonable approach. However, further refinement of the CFA/I fimbriae and/or investigation of an alternate vector could hold promise.

ABSTRACT

The Protective Role of Human Mesenchymal Stem Cells in Acute Pancreatitis

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Sponsored by: Tien C. Ko, MD, Yanna Cao, MD, Department of Surgery

Supported by: Volunteer

Key Words: Human mesenchymal stem cells, mouse model, acute pancreatitis

Introduction. Administration of human mesenchymal stem cells (hMSCs) can beneficially modulate the host immune response against various diseases and improve the survival rate and preserve or restore organ function. hMSCs have been successfully given to humans for the treatment of graft-versus-host disease and appear to be potent immunomodulators. However, whether hMSCs have beneficial effects on an inflammatory disease such as pancreatitis is largely unknown. Therefore, the objective of this study is to use an animal pancreatitis model to evaluate hMSCs' therapeutic effects and further investigate the mechanism. **Experimental Design and Methods.** In mouse acute pancreatitis (AP) model, C57BL/6 mice (male, 8 wks old) were divided into 3 groups: control (with PBS, n=2), AP (with cerulein and PBS, n=6), AP+hMSCs (with cerulein and hMSCs, n=6). Cerulein was given as 12 hourly ip injections (50 µg/kg) while the same amount and frequency of PBS was injected ip for control. The hMSC (Lonza, 1 million/0.1 ml) or PBS was injected via tail vein at 24 and 48 hours following the first cerulein injection. The mice were euthanized 4 days after the first cerulein injections. The pancreas was harvested for H&E staining and histological assessment on AP score criteria (including edema, necrosis and inflammation) and CP score criteria (including areas of acinar injury and fibrosis). **Results.** There are marginal differences in AP scores among the groups of control, AP, AP+hMSCs (2.5, 6.1, 6.0, p=0.05), and no differences in CP-fibrosis scores (0, 1.7, 1.0, p=0.12). However, there are significant differences in acinar injury scores (0, 7.3, 3.2, p<0.001). **Conclusions.** hMSCs treatment after AP induction attenuated acinar injury shown by the decreased acinar injury scores. Our data suggest that hMSCs in the AP mouse model may accelerate acinar regeneration after AP induction, which provides further insights on the protective role of hMSCs in AP. Future experimentation is required for the underlying mechanistic studies that may ultimately lead to the development of therapeutic strategies using hMSCs in AP and in other inflammatory diseases.

ABSTRACT

PPAR γ Mediates Glutamine's Protection of the Post-ischemic Gut

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Sponsored by: Rosemary Kozar, MD, PhD, Department of Surgery

Supported by: National Institute of Diabetes and Digestive and Kidney Disease, 5T35DK7676-20

Key Words: PPAR γ , Glutamine, Ischemia-Reperfusion

PPAR γ is a nuclear transcription factor and a key regulator of inflammation. Previous studies in our lab have shown that the administration of enteral glutamine during mesenteric ischemia/reperfusion (I/R) provides a protective effect to the gut and that glutamine increases the activity of PPAR γ . To further confirm the relationship between PPAR γ and glutamine, intestinal specific conditional PPAR γ knock-out (KO) mice were used to test the hypothesis that PPAR γ mediates the protective effects of enteral glutamine during intestinal ischemia-reperfusion (IR). In a mouse model of IR, wild type (WT) and intestinal specific PPAR γ KO mice were subjected to one hour of mesenteric ischemia followed six hours of reperfusion and compared to shams (n=5/group). Enteral glutamine was administered into the small bowel at the onset of reperfusion, a clinically relevant model. Intestinal tissue was harvested and evaluated for injury by histopathology and apoptosis, and for inflammation by assessing myeloperoxidase (MPO) activity and neutrophil infiltration. Intestinal injury and inflammation were increased in WT mice compared to shams and further increased in KO mice compared to shams after IR. Injury and inflammation were mitigated by glutamine in WT but not in PPAR γ KO mice. In conclusion, using intestinal specific conditional PPAR γ KO mice, we further confirmed that PPAR γ mediates the protective effects of enteral glutamine in the post ischemic gut.

ABSTRACT

Human Induced Pluripotent Stem Cells-derived neuronal-restricted precursors for Spinal Cord Injury

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Supported by: National Institute of Neurological Disorders and Stroke, T35 NS 064931-3

Key Words: SCI, Inducible Pluripotent Stem Cells, Embryonic Stem Cells

Human inducible pluripotent stem cells (iPSC), the remarkable pluripotent embryonic stem cell (ESC)-like cells reprogrammed from embryonic or adult somatic cells by over-expression of four developmental/pluripotency transcription factors, offer tremendous potential for individualized patient- and disease-specific therapy. Transplantation of human iPSC-derived neural progenitor cells (NPC) could be one of the most promised novel reparative strategies to promote functional recovery after spinal cord injury (SCI). However, one of the major challenges to fully realize the full therapeutic potential of iPSC for SCI and other neurological diseases is to direct human iPSC to differentiate into desired NPC in vitro and then purify these cells before transplantation. In this study, we first obtained fibroblasts from the skin biopsies of spinal cord injury patients. We then developed SCI patient-specific iPSCs by overexpressing four transcript factors, sox2, c-myc, Oct4 and Klf4 in fibroblasts using retroviral method. Human iPSC expressed all undifferentiated hESC markers suggesting that it is fully reprogrammed. We then induced these iPSC into neural differentiation using our established neural differentiation protocol. Human iPSC differentiated into neural stem cells expressing sox1 and pax6, the two early neural stem cell markers, at 15 days post-differentiation (PD) and into neuronal precursor cells (NRP) expressing β -tubulin III and nestin at 26 days PD. At this stage, many β -tubulin III⁺ cells also expressed a membrane antigen, A2B5. We purified A2B5⁺ NRP by fluorescence-assist-cell sort using A2B5 antibody. The purified cells were all expressed β -tubulin III without contamination of other types of cells including undifferentiated iPSC. These cells can be proliferated in vitro in the presence of mitogens and differentiate into mature neurons after further neuronal differentiation for 10 days in vitro. NRPs were labeled by expression of GFP through retroviral infection in vitro and then transplanted into the injured spinal cord of severe-combined immunodeficiency (SCID) mice at 8 days after moderate contusion. Robust survival of grafted NRPs was observed in the injured spinal cord weeks after transplantation. The transplanted NRPs differentiated into mature neurons express NeuN and graft-derived neurons sent processes into gray matter and formed connections with the host neurons. Thus, we establish an in vitro protocol to direct hiPSC to differentiate into neuronal precursor cells (NRPs) and develop a FACS method to purify NRPs. Purified NRPs can differentiate into mature neurons in vitro and survive and mature into neurons after transplantation into the traumatically injured spinal cord. These results suggest that human iPSC-derived NRPs have great therapeutic potential for neuronal replacement after SCI and other neurological diseases.

ABSTRACT

The Importance of Phosphorylation in Regulating Myosin Phosphatase Target Subunit 1 in Ileus

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Sponsored by: Karen Uray, PhD, Department of Pediatric Surgery

Supported by: National Institute of Diabetes and Digestive and Kidney Disease, 5T35DK7676-20

Key Words: MYPT1, MLC, ileus

Ileus, intestinal dysmotility, often occurs after trauma, preventing enteral feeding, prolonging hospital stays, and increasing morbidity. The mechanism of pathogenesis is unknown for intestinal edema which can lead to ileus. The goal of my research is to understand the mechanism by which ileus develops in trauma patients with the aim of identifying therapeutic targets to treat ileus. Intestinal edema contributes to ileus by decreasing contractile activity. Smooth muscle contraction is controlled by phosphorylation of myosin light chain (MLC). Edema decreases phosphorylation of MLC leading to decreased contractile activity. MLC phosphatase activity is increased in edema. MLC phosphatase activity is regulated by inhibitory phosphorylation of the myosin phosphatase target subunit 1 (MYPT1). Intestinal edema shows decreased phosphorylation of MYPT1. MLC phosphorylation is controlled by a complex kinase cascade including P21-activated kinase (PAK). My hypothesis is that P21-activated kinase (PAK) signals through the myosin binding subunit (MYPT1) of myosin light chain (MLC) phosphatase to regulate MLC phosphorylation. By creating specific mutations in MYPT1, phosphorylation sites at threonine 696 and threonine 853 have been removed and replaced with an alanine. The mutations have been made in MYPT1 and are being used to transfect human intestinal smooth muscle cells. The cells are then lysed and analyzed by western blots. The mutation at Thr853 decreased MYPT1 phosphorylation at both sites. We hope to determine the necessity of MYPT1 phosphorylation at one or both inhibitory sites in mediating the regulatory effects of PAK on MLC phosphorylation.

ABSTRACT

Determining the Association between Cirrhosis and Coagulopathy Using Thrombelastography (TEG) in Trauma Patients

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Class of 2015

Sponsored by: John B. Holcomb, MD, Department of Surgery and Director of the Center for Translational Injury Research

Supported by: The Center for Translational Injury Research (CeTIR)

Key Words: Cirrhosis, trauma, coagulopathy, thrombelastography (TEG)

Background: Patients with liver cirrhosis experience abnormal synthesis of coagulation factors that regulate blood clotting and clot lysis, and have increased mortality secondary to hemorrhage after injury. Plasma transfusion is commonly initiated in these patients. It is thought that conventional coagulation tests (CCTs) such as PT and aPTT are limited in their clinical utility as they are not functional measures of coagulation. TEG, however, functionally measures coagulation, lysis, and platelet function, which may be more clinically useful in this difficult to manage patient population. The purpose of this retrospective study was to describe TEG results in cirrhotic trauma patients and suggest how these results might be useful to clinicians.

Methods: A TEG database of 3279 trauma patients admitted to Memorial Hermann Hospital from August 2009 through February 2012 was used for this study. Patients with cirrhosis were identified by medical history, CT scans, and laboratory values that met previously published criteria. Mann-Whitney statistical analysis was used to compare TEG and CCT values between the cirrhotic and non-cirrhotic groups, and p-values <0.05 were accepted as significant.

Results: A total of 80 patients met the criteria for this study. The cohort was 75% male with a median age of 56 years. The mortality rate was 30% for the cirrhotic group (median ISS 18), compared to 14% for the entire database (median ISS 17). The cirrhotic group demonstrated prolonged median PT and PTT times, an elevated INR, and a significantly lower platelet count when compared to all trauma patients. In the cirrhotic group, there was no difference in ACT or r-values, indicating maintained plasma protein function, but they had a significantly reduced MA (p-value <0.001) and G-value (p-value <0.001), indicating reduced clot strength secondary to reduced platelet or fibrinogen function. Abnormal CCTs and TEG corresponded with transfusion, as 79% of cirrhotic patients received blood and 36% received ≥ 10 units within 24 hours of admission. Corresponding rates in all trauma patients were 29% and 6%.

Conclusion: Cirrhotic patients demonstrated abnormal CCTs and TEG values that were consistent with increased transfusion requirements and higher mortality. TEG analysis suggests that cirrhosis results in reduced functional clot strength due to deficits of fibrinogen and/or platelet function, supporting that earlier use of these products may be indicated when transfusion is required. TEG is a rapid and valuable predictor of coagulopathy in cirrhotic trauma patients, and clinicians should consider using it to direct treatment strategy.

ABSTRACT

Title Assessment of Local and Systemic Aerobic Thresholds with Near-Infrared Spectroscopy

BRAIDEN M. HEAPS The University of Texas at Houston Medical School Class of 2015

Sponsored by: Catherine Ambrose, PhD, Department of Orthopedic Surgery

Supported by: Catherine Ambrose, PhD, Department of Orthopedic Surgery; University of Texas at Houston Medical School – Office of the Dean

Key Words: NIRS, Lactate, Threshold, Performance

Aerobic thresholds represent the exercise intensity where anaerobic metabolism begins to operate, and lactate starts to accumulate in the blood stream. They are valuable measurements for endurance athletes because they can be used to determine exercise intensity while training. Aerobic thresholds have long been evaluated by sampling blood for lactate concentrations. This study was designed to determine if a new near-infrared spectroscopy (NIRS) sensor could be used as an alternate to blood sampling for accurately predicting aerobic thresholds non-invasively. Forty runners of varying training levels were asked to perform a graded exercise on a treadmill. A very light initial workload was set at each subject's leisure, and the pace of the following stages were increased by 20 seconds per mile every 3 minutes. NIRS, respiratory gases and heart rate data were sampled throughout the exercise from each participant every 3 seconds. Blood samples were collected at the end of each running stage, and the lactate threshold was determined with the V-slope method, which is the standard method. Preliminary results indicate a very good correlation between NIRS data and oxygen uptake (VO₂). In several subjects, muscle desaturation, from the NIRS sensor, was found to begin in correspondence with lactate threshold, from the blood sampling. Data collection is ongoing. Definitive results will be determined after extensive biostatistical analysis.

ABSTRACT

Review of the First Year of a Prospective Study on the Effects of a Redefined Post-fracture Care Protocol for Metabolic Bone Disease

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Sponsored by: Catherine Ambrose, PhD, Department of Orthopaedic Surgery
Milan Sen, MD, Department of Orthopaedic Surgery

Supported by: Catherine Ambrose, PhD, Department of Orthopaedic Surgery; University of Texas at Houston Medical School – Office of the Dean

Key Words: metabolic bone disease, vitamin D deficiency, FRAX, fragility fracture

Fragility fractures are common complications of metabolic bone disease and will be experienced by approximately 50% of women and 25% of men over age 50. The occurrence of a fragility fracture results in a 50% increased risk of a second fracture, and an increased risk of premature death. Therefore it is imperative that effective protocols be implemented to ensure the early detection and treatment of metabolic bone disease as well as fragility fractures. The current protocol at our institution has been implemented for 1 year and includes the referral of fragility fracture patients to the endocrinologist or geriatrician on call for a full metabolic assessment. Our hypothesis is that the new protocol has led to increased frequency of detection of metabolic bone disease, heightened patient awareness of their diagnosis, and that continued medical treatment was obtained for the disease. This research protocol has been approved by our IRB as protocol HSC-MS-10-0291. Information was retrieved from patient's health records at Memorial Hermann Hospital and from phone surveys to determine metabolic status and FRAX score. Preliminary data collection revealed a total of 58 subjects (17 males, 41 females) with an average age of 76. The average BMI was 26.1 and 24% of subjects had a BMI over 30. The most frequent fracture locations were proximal femur (41%) and distal femur (16%). Vitamin D deficiency was present in 19% of male patients and 38% of female patients; 42% were between the ages of 80 and 89 years old. Data collection is ongoing.

ABSTRACT

Development of a Protocol to Establish a UT Houston Health Science Center (UTHSC) Fecal Transplant (FT) Center

LY HOANG

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Sponsored by: Herbert L. DuPont, MD, Center for Infectious Diseases

Supported by: Herbert L. DuPont, MD, Center for Infectious Diseases; University of Texas at Houston Medical School – Office of the Dean

Key Words: Fecal transplantation

With the increasing frequency of *Clostridium difficile* infection (CDI) and rising mortality rate and failure of current forms of treatment for recurrent CDI, alternative methods of management are being sought. Since disruption of colonic bacterial flora is a prerequisite for developing CDI, research centers have worked with probiotics and administration of feces from a family member or donor into the gastrointestinal tract of affected patients. This study is designed to 1) conduct a survey to determine the number of recurrent *C. difficile* infection in Houston and the number of patients likely to be referred to a FT center, and 2) review the literature to develop a model protocol for development of a FT center at UT Houston. Two hundred sixty-four surveys (187 to gastroenterologists [GI] and 77 to infectious diseases [ID] specialists) were distributed. The survey's response rate was 34% (89 completed/returned). Fifty-five (29%, 55/187) valid forms received were from GI physicians and 32 (42%, 32/77) from ID specialists (Table). The GI and ID physicians who responded were equally supportive of a local FT center and expressed interest in using FT for therapy of their patients with recurrent CDI (35/55=64% for GI physicians and 22/32=69% for ID physicians, $p=0.628$). Sixteen percent of ID physicians (5/32) responding and 7% (4/55) of GI physicians responding did not see a need for the center ($p=0.217$). A majority of responding gastroenterologists (49/55, 89%) and 26 Infectious diseases specialists (26/32, 81%) indicated that they would refer patients to a newly developed local FT center. Moreover, 994 studies were found on PubMed using the search words fecal transplantation, bacteriotherapy, and *Clostridium difficile*. 28 studies were found to meet the inclusion criteria of original published research with patient resolution of $\geq 70\%$. Data analysis showed that the preferred method of infusion is colonoscopy (46%, 12/26), enema (27%, 7/26), and nasogastric (15%, 4/26). Most preparations contained 0-50 g of stool (46%, 12/26) and utilized 100-500 mL of infused suspension (42%, 11/26). The majority (81%) of donor-patient relationships falls in the category of family, spouse, or volunteer. Only 5 studies (19%) were purely volunteer based. In conclusion, there is an interest for a FT center in Houston that can be met with the development of an appropriate protocol.

ABSTRACT

Effect of bile salts on rifaximin treatment of enteroaggregative *E. coli* in an *in vitro* biofilm model of intra-intestinal infection compared to planktonic cells

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Sponsored by: Heidi B. Kaplan, PhD, Department of Microbiology and Molecular Genetics

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: biofilm, EAEC, enteroaggregative *Escherichia coli*, rifaximin

Enteroaggregative *Escherichia coli* (EAEC) are a major cause of severe and often lethal pediatric diarrhea in developing countries and of persistent diarrhea in developed countries. EAEC infections are difficult to management with oral rehydration therapy alone. The ability of EAEC to adhere to intestinal mucosal tissue, forming a biofilm, has been linked to its therapeutic resistance, which is a hallmark of biofilm infections. We hypothesized that exposure of EAEC biofilms cells to bile salts (sodium cholate [SC] and sodium taurocholate [ST]) during antibiotic treatment with rifaximin might render the drug more effective. EAEC cells were grown in an *in vitro* model, in which growth under varying conditions of bile salts and rifaximin was quantified, for both planktonic and biofilm cells. The minimum inhibitory concentration (MIC) of 8 µg/mL of rifaximin was measured for planktonic EAEC and HS, a non-biofilm forming *E. coli*, under conditions in which the drug was optimally solubilized. However, an MIC of 64 µg/mL was observed for poorly solubilized rifaximin. Physiologically relevant concentrations of SC and ST were used (0 and 50 mM) and it was determined that addition of SC and ST reduced planktonic growth by as much as 10 and 5 fold, respectively at 50 mM. To examine the effect of SC and ST on biofilm-grown EAEC cells, biofilms were grown for 48 hrs, and exposed to each bile salt for 24 hours, and the biofilm growth was quantified using a standard crystal violet biofilm assay. Exposure of the biofilm cells to SC and ST reduced growth by more than 3 fold at 50 mM of SC or ST. The same experiment was carried out with biofilms grown for 48 hrs and exposed to rifaximin with and without SC and ST. Exposure to rifaximin alone provided no MIC, as the cells remained resistant to concentrations up to 64 µg/ml regardless of the drug solubilization. A synergistic effect on biofilm growth was observed when rifaximin and bile salt were combined. There was maximum sensitivity of biofilm growth at concentration as low as 10 mM of SC and 8 µg/ml of rifaximin. In conclusion, our hypothesis proved correct that the presence of bile salts improved the efficacy of rifaximin for planktonic and biofilm EAEC cells.

ABSTRACT

Diffusion Tensor Imaging Changes Correlate with NIH Stroke Scale Scores for Patients with Intracerebral Hemorrhage

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Sponsored by: Nicole Gonzales, MD, Department of Neurology

Supported by: National Institute of Neurological Disorders and Stroke, T35 NS 064931-3

Key Words: Diffusion tensor imaging, fractional anisotropy, intracerebral hemorrhage

Background: Intracerebral hemorrhage (ICH) is the most devastating form of stroke and currently lacks viable treatment options. The SHRINC study (Safety of Pioglitazone for Hematoma Resolution In Intracerebral Hemorrhage) is a phase 2 clinical trial testing the safety of pioglitazone (PIO) to promote hematoma resolution by increasing the activity of the body's resident phagocytes against red blood cells. Diffusion tensor imaging (DTI) records fractional anisotropy (FA), which measures the degree of directionality in the diffusion process of water. Mean diffusivity (MD) measures the amount of diffusion. Disintegration of the cortical spinal tracts (CST) is represented by a lower FA value and higher MD value. We hypothesize that MD, FA, and volume of the structures containing CST will correlate with motor outcome.

Methods: We have obtained serial MRI scans of patients enrolled in SHRINC between day 21 and day 70 after ICH with a DTI sequence on a 3-Tesla Phillips scanner. Using ImageJ software we obtained FA and MD data on the CST by drawing a region of interest (ROI) on the cerebral peduncle, internal capsule, and pons both ipsilateral and contralateral to the hematoma. The ROIs were used to calculate FA, MD, and volume. The correlation of FA, MD, and volume with NIHSS scores were evaluated by Spearman partial rank order controlled for time.

Results: We obtained data on 7 patients with basal ganglia hemorrhages, 43% male. Median GCS was 15 and baseline median hematoma volume by CT was 30cc (IQR 14, 33). Preliminary analysis demonstrates that lower FA values correspond to higher NIHSS scores and increased motor deficits. On days 28 and 42 FA of the cerebral peduncle on the affected side had an inverse correlation with NIHSS scores with correlation coefficients of 0.77 and 0.80 respectively. Additional data is needed to investigate further.

Conclusions: ICH is a devastating disease with no approved medical therapies. These data may be an important biomarker that allows clinicians to monitor patient recovery and provide insight on the pathophysiologic course of this disease. When the pathophysiology is better understood, potential treatment can be tailored to those patients most likely to respond.

ABSTRACT

Identification of DsbA and DsbB proteins of *Corynebacterium diphtheriae*

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Sponsored by: Hung Ton-That, PhD, Department of Microbiology and Molecular Genetics

Supported by: Hung Ton-That, PhD; University of Texas at Houston Medical School – Office of the Dean

Key Words: pili, disulfide bonds, secreted proteins

Disulfide bonds are post-translational modifications made to exported proteins that assist in their folding and stability. A well-studied system in *Escherichia coli* and other gram-negative organisms, the disulfide bond-forming (Dsb) systems introduces disulfide bonds into secreted proteins. However, equivalent systems are not well described in Gram-positive bacteria, specifically *Corynebacterium diphtheriae*, the causative agent of diphtheria known to assemble disulfide bond-containing pili that are attached to the bacterial peptidoglycan and involved in bacterial pathogenesis. Using SpaA pili as an experimental substrate, this project focuses on identifying the DsbA and DsbB proteins of the Corynebacterial Dsb pathway. DsbA is an oxidoreductase that introduces disulfide bonds into secreted proteins, and DsbB is a membrane-bound protein that re-oxidizes DsbA. By bioinformatics analysis and homology searches, we identified five Dsb-like proteins with a CXXC motif, which is a unique feature of Dsb proteins. To determine whether these proteins are involved in pilus assembly, we generated four individual deletion mutants of genes encoding these Dsb-like proteins, namely *dsb1-4*. Pilus assembly was analyzed by immunoblotting and immuno-electron microscopy with specific antibodies against SpaA. While deletion of *dsb2*, *dsb3*, and *dsb4* did not affect pilus assembly, deletion of *dsb1*, which predictably encodes DsbA, diminished the formation of SpaA pili on the bacterial surface (as evidenced by immunoblotting), similar to mutations of two cysteine residues forming the disulfide bond within the SpaA pilin. Current work aims to generate deletion mutants of *dsb5*, which potentially encodes DsbB based on several conserved features such as the CXXC motif and multiple transmembrane domains.

ABSTRACT

Preprocessing, registration and statistical analysis of anatomical MRI and diffusion tensor imaging in subjects with mild traumatic brain injury

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Supported by: David Gorenstein, PhD and David Volk, PhD, Institute for Molecular Medicine; University of Texas at Houston Medical School – Office of the Dean

Key Words: pili, disulfide bonds, secreted proteins

The use of advanced magnetic resonance imaging (MRI) techniques to determine structural, morphometric, and myelin changes in the brain due to mild traumatic brain injury (mTBI) was investigated. Fifty two mTBI subjects (40 male and 12 female) were scanned within 24 hours of the traumatic event, and a follow-up scan 3 months later. Thirty three orthopedic controls (23 male and 10 female) were also scanned at baseline and three months later. Fractional anisotropy (FA), parallel diffusivity (L1), perpendicular diffusivity (LT), and mean diffusivity (MD) derived from diffusion tensor imaging (DTI) were calculated as potential biomarkers to assess structural changes caused by the traumatic event. Jacobian determinant images derived from high contrast, structural T1 weighted images, were used to evaluate global and regional volumetric changes. The volumetric changes were determined using tensor based morphometry (TBM). All images were non-linearly registered to an unbiased template. Voxel-wise differences in FA, L1, LT, MD, and Jacobian determinants between controls and mTBI patients was performed using Standard Parametric Mapping (SPM5). Region-of-interest (ROI) analysis was also performed to compare regional changes in FA, L1, LT, and MD. Statistically significant differences in any of these measures between mTBI and controls were not observed. However, multiple trends have been noted. Such trends include an increased FA in white matter regions in mTBI subjects relative to controls in the acute phase that decreased with time. Another noted trend is the increase in parallel diffusivity in internal capsule and corona radiata regions. Finally, analysis has shown an increase in volume in certain white matter areas of the corona radiata and internal capsule following the traumatic event. These trends may be the result of acute inflammation caused by diffuse axonal injury and axonal swelling. Despite the current lack of statistically significant results, these trends provide insight into potential brain injury biomarkers.

ABSTRACT

A double blind, randomized, placebo-controlled study to investigate the effectiveness of IV acetaminophen administered during functional endoscopic sinus surgery in reducing the use of opiates to treat postoperative pain

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Supported by: National Institute of Diabetes and Digestive and Kidney Disease, 5T35DK7676-20

Key Words: Acetaminophen, Functional endoscopic sinus surgery (FESS), opiates, pain

Specific Aims: The primary aim of this study was to assess the efficacy of IV acetaminophen in controlling postoperative pain after functional endoscopic sinus surgery (FESS). Secondary to this was assessing the impact of IV acetaminophen on recovery by monitoring the need for pain control by opiates & any changes in vital signs. Additionally, we analyzed patient satisfaction through a 12 hour and 24 hour postoperative survey.

Background: Pain has long been known to be associated with poor outcomes following surgical procedures. For patients with Chronic Rhinosinusitis (CRS), endoscopic surgery is often the only means of treatment available. Pain is currently managed intra- & postoperatively by a host of opioid analgesics that carry a host of undesirable side effects including decreased GI motility, bladder dysfunction, cardiac effects, immune modulation, and high levels of sedation. Opiates have also been shown to hinder postoperative recovery. For this reason, novel methods for pain control are essential to improving the standard of care for the surgical patient.

Methods: A double blind, prospective randomized controlled study at a single university-affiliated institution was used to investigate our aims. Patients were approached in clinic after scheduling FESS for management of CRS. These patients were screened for exclusion criteria such as a history of liver or kidney disease, a recent history of opioid pain control, or any history of morphine intolerance. We established a baseline pain score using a Visual Analog Scale (VAS) with grading from 0-100 prior to surgery. The patient received either 1000mg IV acetaminophen or a comparably delivered saline placebo at least 15 minutes prior to the start of the procedure. Strict anesthesia parameters were adhered to with careful documentation of any additional fentanyl given. Episodes of tachycardia and hypertension were also documented intraoperatively. Four hours after the initial dose, a second and equal dose of 1000mg IV acetaminophen or saline placebo was given whether intraoperatively or in recovery. Upon completion of the procedure and transport to the Post-Anesthesia Care Unit (PACU), we began a postoperative assessment of vital signs, level of sedation, & pain according to the VAS. These

assessments were made every 15 minutes for the first hour of recovery, and every subsequent hour thereafter. Upon discharge, the patient was given careful instruction regarding their at-home care along with an additional two 500mg tablets of oral acetaminophen & 325mg tablets of hydrocodone-acetaminophen as rescue analgesics. The patient was also given the 12 and 24-hour questionnaire to complete. A member of our research team contacted the patient at 24 hours after discharge to discuss the results of their satisfaction questionnaire.

Results: With a desire to enroll 60 patients, 30 patients in each of the acetaminophen group and placebo group, this project will exceed the 10 week span of the UT Summer Research Program. Two patients have been enrolled to-date and both have tolerated the investigational drug very well. It is unfitting to assign any results at this time until more data is collected.

Conclusion: Although we do not have data at this time to support any broad conclusions, our questionnaire results from both patients have been very positive. They have each felt their participation in the study improved their care. There have been no adverse reactions in either patient.

ABSTRACT

Quality Improvement in the Pediatric Operating Room

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Sponsored by: Maria Matuszczak, MD, Department of Anesthesiology

Supported by: Maria Matuszczak, MD, Department of Anesthesiology; University of Texas at Houston Medical School – Office of the Dean

Key Words: Quality Improvement, Pediatric, Airway, Equipment

Quality improvement projects are critical in the management of the operating room, enabling us to assess and improve the safety and efficiency of the OR. To further its goal of improving patient safety, the Pediatric Operating Room Safety and Quality Council decided to focus its efforts on promoting consistent stocking and timely replacement of operating room equipment. Missing equipment places patients at risk and interrupts the smooth function of the operating room by preventing the prompt start and progression of procedures. To assess the problem of inadequate stocking, three audits of the anesthesia carts in our pediatric operating rooms were undertaken in December 2011 and summer 2012. The audits demonstrated deficiencies in our inventory, turnover processes, and overnight re-stocking that, when addressed, resulted in improved equipment availability in our operating rooms.

ABSTRACT

Sevelamer is More Cost-Effective than Calcium-based Phosphate Binders in ESRD Patients: A Comparative Cost-Effectiveness and Sensitivity Analysis

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Sponsored by: Donald Molony, MD, Dept. of Internal Medicine, Renal Diseases Division

Supported by: National Institute of Diabetes and Digestive and Kidney Disease, 5T35DK7676-20

Key Words: cost-effectiveness, phosphate binders, end stage renal disease

Background: Managing end stage renal disease (ESRD) is very expensive and much of its cost is attributable to medications. Thus, the comparative cost-effectiveness (CE) of the most commonly used phosphate binders, sevelamer and calcium salts - calcium carbonate (CC)/ acetate (CA), is an important question. We chose to evaluate CE of each phosphate binder and then performed a sensitivity analysis to determine which factors most importantly influence the CE relationships.

Methods: We performed the CE analysis by incorporating important costs and savings - medication costs, hospital days, and cardiovascular surgeries. We conducted a systematic review of the literature to perform a meta-analysis (reported elsewhere) and constructed an economic model to calculate the incremental cost-effectiveness ratios (ICER) of sevelamer vs. CC/CA using published mortality and hospitalization rates. We performed a sensitivity analysis by changing the relative contribution of hospitalization and dosages across the range of survival benefits.

Results: In incident ESRD patients, when comparing sevelamer to CC, the ICER is \$36,485.53 and when comparing sevelamer to CA, the ICER is \$28,428.62/patient year saved. In a prevalent ESRD population, the ICERs were \$46,674.79 and \$36,367.84/patient year saved for sevelamer vs. CC and vs. CA, respectively. In a sensitivity analysis, varying the hospital-days-saved from the 1.6 days per year of life savings observed with sevelamer vs. calcium binders in the DCOR trial, we demonstrated that a reduction in hospital days of greater than 1 for incident patients and of > 2 for prevalent patients will produce ICERs of less than the standard accepted CE threshold of \$50,000 also.

Conclusions: The current CE analysis demonstrates that sevelamer is a cost-effective phosphate binder compared to the calcium salts. Even with a higher cost, sevelamer proves to be cost-effective primarily because of a significantly lower mortality rate, lesser number of hospital days, and reduced cardiovascular surgeries, all perhaps attributable to lower vascular calcification. The potential quality of life (QOL) benefits from sevelamer vs. calcium based phosphate binders could not be tested in this model because changes in QOL have not been measured in the reported clinical studies. Nonetheless, we will include a QOL discounting in our final model.

ABSTRACT

Onychomades is due to newly described Coxsackie virus subtype (CVA6)

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Sponsored by: Adelaide Hebert, MD, Department of Dermatology

Supported by: Volunteer

Key Words: Onychomadesis, Coxsackievirus A6, hand foot & mouth disease, vesicles

A 16 month old African American male infant developed vesicles of both the hands and feet, as well as erosions of the oral mucous membranes with fever and onychomadesis. Recurrent swelling in the fingers and toes occurred, along with recurrent, painful mouth ulcers. The nipples had tender, oozing superficial erosions. Onychomadesis recurred for 2 cycles with nail re-growth and shedding. The patient's nails also became distally dystrophic with horizontal medium brown bands near the distal nail plate. The general health of the child had previously been good. Initially, clinical findings included fever, with the cutaneous and mucosal lesions appearing on the upper inner surface of the lip, and the fingers and toes 1 to 2 days later. Clinical diagnosis of Hand, Foot, and Mouth Disease was made based on the physical findings and time course of the illness. The patient was admitted to Memorial Hermann Children's Hospital subsequent to the resolution of the initial cutaneous vesicular eruptions due to the fever and unusual cutaneous and nail manifestations. A viral culture of serum via RNA PCR was performed at the time of hospital admission to isolate and identify the viral strain. The nail findings in this patient suggest the phenotypic expression described in patients infected by Coxsackievirus subtype A6. The clinical findings in a child diagnosed with Hand, Foot, and Mouth Disease from the newly described Coxsackievirus A6 will be emphasized.

ABSTRACT

Effect of Bile Duct Ligation on *E. faecalis* Colonization of the Small Intestine in Indomethacin-treated Rats

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Sponsored by Lenard M. Lichtenberger, PhD, Department of Integrative Biology and Pharmacology

Supported by: National Institute of Diabetes and Digestive and Kidney Disease, 5T35DK7676-20

Key Words: NSAID, bile acid, *E. faecalis*, indomethacin

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat pain and inflammation, but are limited in their success by their ability to cause damage to GI mucosa. NSAID-induced ulceration to the GI mucosa allows for overgrowth of normal gut bacteria and increases the probability of their dissemination throughout the body. Presence of *Enterococcus faecalis*, a normal inhabitant of the intestine but also a common cause of nosocomial infections, appears to increase dramatically in the rat gut with use of an NSAID. Although an association between gut injury and bacterial overgrowth has been shown previously, no causative effect has been established between the two. Current research has shown that restricting flow of bile while using an NSAID has been shown to lessen ulceration and injury. In fact, *E. faecalis* grows in bile, which could be a possible explanation for the augmented damage during presence of bile in the GI lumen.

Hypothesis: Our current hypothesis is that restricting bile in the presence of an NSAID will not only reduce intestinal injury but also control overgrowth of *E. faecalis*.

Methods: In this study, Sprague-Dawley rats with bile duct ligation (BDL) and sham-operated (SO) rats were given two daily subcutaneous injections of saline or 7.5mg/kg of indomethacin, an NSAID, starting 24 hours after surgery. Rats were then sacrificed 48 hours post-dosing. The distal small intestine, liver, kidney, and feces samples were homogenized and plated on bile esculin azide agar (BEA), an agar selective for *Enterococcus* species, to determine the degree of bacteria present. Total bile acid content was measured to confirm that BDL was successful and fecal hemoglobin was measured to assess GI bleeding.

Results: Rats that were sham-operated were treated with indomethacin experienced a daily increase in excreted hemoglobin, whereas BDL rats treated with indomethacin showed a minimal and comparable amount of fecal hemoglobin to the SO/saline and BDL/saline rats, indicating that the BDL helped attenuate intestinal injury. In addition, *E. faecalis* colonies were significantly reduced in BDL/saline rats ($p < .0095$) and greatly increased in SO/indomethacin rats when compared to SO/saline rats ($p < .0031$). The BDL/indo rats had similar levels of *E. faecalis* as the SO/saline rats. Of the four groups, only the SO/indomethacin rats demonstrated both gut injury and bacterial overgrowth. Thus, BDL helped prevent both injury and *E. faecalis* overgrowth in the GI tract.

Conclusion: Bile plays an important role in NSAID injury and *E. faecalis* growth in the intestine. As NSAIDs become more extensive in treatment applicability, understanding the relationship between *E. faecalis*, bile, and NSAIDs may be the key to preventing unnecessary GI injury.

ABSTRACT

Differential Immune Response to Mycobacterial Glycolipid in the Induction of Tuberculosis-Related Pathology

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Sponsored by: Jeffrey K. Actor, PhD, Department of Pathology and Laboratory Medicine

Supported by: National Institute of Diabetes and Digestive and Kidney Disease, 5T35DK7676-20

Key Words: Tuberculosis, TDM, Inflammation, Hypersensitivity

Background/Significance: *Mycobacterium tuberculosis* (*Mtb*) is the etiological agent of tuberculosis (TB). Trehalose 6,6'-dimycolate (TDM), an abundant glycolipid on the *Mtb* cell surface, functions as a key mediator of pathogenesis. Therefore, a targeted immune response towards TDM may be critical for development of protective host immunity. Current immunization efforts utilize the bacille Calmette–Guérin (BCG) vaccine, a live attenuated strain of *M. bovis*. However, efficacy in adults is limited, particularly in preventing pathological damage and transmission of disease.

Purpose: Here we aim to determine if there are unique qualitative differences in acute and hypersensitive immune responses to TDM derived from virulent *Mtb* versus TDM from the *M. bovis* vaccine strain.

Methodology: Hypersensitive Model: BALB/c mice were subcutaneously immunized with *Mtb* TDM (50µg, base of the tail), and intravenously challenged 2 weeks later with *Mtb*- or *M. bovis*-derived TDM emulsion formulated in Drakeol/Tween-80. Acute Model: Mice were similarly challenged with either TDM species, but without prior sensitization, and sacrificed after 6 days. Lungs were weighed, then sectioned to evaluate pathology and cytokine production. Data obtained was compared across groups, and against naive mice or mice challenged with emulsion vehicle alone, then analyzed by an unpaired *t*-test or two-way ANOVA; differences between means were significant at a level of $p \leq 0.05$.

Results: Mice acutely challenged with *M. bovis* TDM showed greater inflammation histologically compared to mice given only *Mtb* TDM, with statistically higher lung-weight indices and levels of pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6 in lung parenchyma. However, more IL-17, IFN- γ and IL-12p40 was evident in the *Mtb* TDM group. Presensitization immunization with *Mtb* TDM followed by challenge with the same glycolipid species, resulted in elicitation of stronger inflammatory responses; average lung-weight indices and cytokines were significantly higher than controls. Histological staining of lung revealed marked lymphocytic infiltration, cuffing of blood vessels, edema and pulmonary obstruction. The cytokine mediators IFN- γ and IL-12 were also significantly elevated. Conversely, immunized mice challenged with *M. bovis* TDM demonstrated inflammatory responses not significantly different from either the acute model, or from emulsion controls. Histological analysis

demonstrated that lung pathology in the BCG challenged group was strikingly similar to the acute model challenge.

Conclusion: The results indicate differences in both acute and hypersensitive immune responses to TDM derived from different mycobacterial strains. Furthermore, development of specific adaptive immune responses to the *Mtb*-derived TDM were demonstrated that had limited cross-reactivity to that of *M. bovis*, thus strongly suggesting the presence of epitopes exclusive to *Mtb* TDM not present on *M. bovis*-derived TDM. Future studies will investigate the novel T cell specific responses to this unique glycolipid.

ABSTRACT

Blunt Traumatic Injury to the Iliac Arteries: Open and Endovascular Outcomes

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Sponsored by: Kristofer Charlton-Ouw, MD, Department of Cardiothoracic and Vascular Surgery

Supported by: Volunteer

Key Words: Iliac arteries, blunt trauma

BACKGROUND: Injury to the iliac arteries and their proximal branches following blunt trauma is uncommon. The 51 cases described in the English literature since 1969 have been presented largely as single case reports or small series of patients. Endovascular repair is becoming the new practice standard and we sought to highlight our outcomes with this new approach.

METHODS: Patient data from 1999-2012 were compiled from the Institutional Trauma Registry, the hospital electronic medical records system, and our departmental outpatient office records. Injuries studied were restricted to the iliac arteries and their 1st and 2nd order pelvic branches. Branches outside the pelvis, such as the terminal gluteal arteries, were excluded. Various outcome indicators, including time spent in the intensive care unit, length of hospital stay, mortality, need for reintervention, and the incidence of post-operative complications were retrospectively assessed.

RESULTS: We identified 110 patients with blunt traumatic injury to the iliac arteries and their proximal branches. Most cases involved the internal iliac artery system with only 12 (11%) involving the external iliac system. 95 (86%) patients received primarily endovascular repair while 7 (6%) received open surgical repair. Of the patients with endovascular repair, 90 (95%) required embolization alone without revascularization. Technical success in controlling pelvic bleeding was achieved in 87 (97%). In-hospital mortality occurred in 43 (39%) of which 38 (88%) had endovascular repair and 3 (7%) had open repair. Cause of death was primarily due to uncontrolled pelvic bleeding in 21 patients (49%) and other associated injuries in 22 (51%).

CONCLUSIONS: Injury to the internal iliac arteries is associated with high mortality mostly due to massive pelvic bleeding and other injuries associated with high-energy blunt trauma. Endovascular embolization techniques have a high technical success rate in controlling pelvic bleeding and have largely replaced open surgical repair.

ABSTRACT

Two New Genes that may promote Thoracic Aortic Aneurysm and Dissection (TAAD)

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Sponsored by: Dianna Milewicz, MD, PhD, Department of Medical Genetics

Supported by: Dianna Milewicz, MD, PhD, Department of Medical Genetics; University of Texas at Houston Medical School – Office of the Dean

Key Words: Thoracic Aortic Aneurysm and Dissection (TAAD), ACTA2, PLEKHO2, PRKG1

Thoracic Aortic Aneurysms and Aortic Dissections (TAAD) are the fifteenth leading cause of death in the United States. Largely asymptomatic, aortic aneurysms can remain undiagnosed until a dissection occurs; therefore, detecting and managing the aneurysms at an early stage is important to prevent sudden deaths. There are three types of TAAD: familial, syndromic, such as in Marfan's syndrome, or sporadic. About 20% of patients with TAAD have been found to have a family history of the disease. The most commonly mutated gene in these families is *ACTA2*, which encodes a smooth muscle-specific α -actin, an essential component of the smooth muscle (SM) contractile unit and the most abundant protein in vascular smooth muscle cells. Interestingly, single point mutations in *ACTA2*, R258H or R258C, predispose individuals to TAAD. Previous data from our lab identified mutations in two additional genes, *PLEKHO2* and *PRKG1*, in families with TAAD using exome sequencing. Similar to the *ACTA2* mutations found previously, these families carried single point mutations in *PLEKHO2* or *PRKG1*, which may have predisposed them to TAAD. We cloned these two genes and expressed them in HeLa and COS cell lines. We also used site-directed mutagenesis to create cell lines expressing the mutated forms of the proteins as seen in the patients. Our next goal is to compare expression of known regulators of smooth muscle cell dynamics in these wild-type and mutant-expressing cell lines.

ABSTRACT

A Comparison Study of Anatomical Femoral Tunnel Placement Anterior Cruciate Ligament Reconstruction

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Sponsored by: Walter R. Lowe, MD, Edward T. Smith Chair in Orthopaedics, Professor and Chairman, Department of Orthopaedic Surgery

Supported by: Catherine Ambrose, PhD, Department of Orthopedics, The University of Texas at Houston Medical School

Key Words: ACL, Femoral Tunnel, Reconstruction, Single Bundle, intraoperative imaging

The long-term outcome of an ACL reconstruction depends heavily on the appropriate graft placement in the most anatomical position possible. An accurate location for drilling of the femoral tunnel, on the medial wall of the lateral femoral condyle during a single-bundle ACL reconstruction, is paramount for achieving a good position with the ACL graft; which in turn results in successful healing. The purpose of this study is to investigate and report on the accuracy and reproducibility of 2 different methods for the placement of the anterior cruciate ligament (ACL) femoral tunnel in its most native location on the femoral condyle. Two different methods are being compared: 1) using intraoperative fluoroscopic imaging and 2) the ruler method. Fresh frozen cadaver knees had pre-op CT images taken, at which point the ACL was removed arthroscopically, and then again reimaged. To test the effectiveness of different methods, a sensor tracking system and custom knee jig were designed to allow for tracking of a 6 DoF positional sensor inside the knee which would track the location on the condyle where the surgeon wanted to place the tunnel. The two methods will be performed by the same surgeon, and then repeated by 10 different surgeons, all on the same knee. Each method is performed according to standard arthroscopic procedures. The positional data is recorded and plotted onto the post-op 3d CT. The quadrant method is then used to measure the distances of the tunnel to be drilled, by the surgeon, away from the native anatomical footprint of the ACL. With the large amount of variables being tested and the time required to construct a protocol for testing, the data is still being collected and analyzed.

ABSTRACT

Neuronal Protection via Endogenous Astrogliosis after Stroke

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Sponsored by: Qi Lin Cao, MD, Department of Neurosurgery

Supported by: Qi Lin Cao, MD, Department of Neurosurgery, University of Texas at Houston Medical School – Office of the Dean

Key Words: Astrocytes, neuroprotection, blood-brain-barrier, stroke, gliosis

Reactive gliosis, which is characterized by the hypertrophic and hyperplastic response of astrocytes, is one of the most commonly occurring phenomena in response to neurological diseases, such as stroke. However, the functions of astrogliosis after stroke have not been well defined. Both beneficial and detrimental effects on functional recovery after neurological diseases have been reported from reactive gliosis. In this study, we have investigated the functions of endogenous astrogliosis and the consequential neuronal survival after ischemic stroke. Our results show astrogliosis plays an essential role in neuroprotection after acute stroke and part of this loss of neurons appears to be mediated by loss of the BBB due to death of the astrocytes.

ABSTRACT

Endovascular Repair of Traumatic Peripheral Arterial Injuries

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Sponsored by: Ali Azizzadeh, MD, Department of Cardiothoracic and Vascular Surgery

Supported by: The University of Texas at Houston Medical School

Key Words: Peripheral artery, trauma, stent graft

Background: Endovascular repair of peripheral arterial trauma is a minimally invasive alternative to open repair in selected patients. The purpose of this study was to evaluate patient outcomes following endovascular repair at an urban level 1 trauma center.

Methods: Patients who underwent endovascular repair of a traumatic peripheral arterial lesion with a stent graft were selected from a prospectively collected institutional trauma registry. Demographic, imaging, operative, and hospital data were also collected through a supplemental review of the patient's records.

Results: Between 8/2004 and 6/2012, 28 patients (20 male, median age 35 years, range 13-88) underwent endovascular repair of a traumatic peripheral arterial lesion. The mean injury severity score was 17.2. The mechanism of injury included gunshot wound (N=12), iatrogenic (N=7), blunt (N=7), and stab wound (N=2). Sites of injury included the subclavian or axillary (N=13), femoral or popliteal (N=9), iliac (N=3), and carotid arteries (N=3). Findings on angiography included pseudoaneurysm (N=10), rupture (N=9), occlusion (N=6), and arteriovenous fistula (N=3). Twenty three patients were treated with a single stent graft while five required one or more extensions (Viabahn, WL Gore & Associates, Flagstaff, AZ). The immediate technical success rate was 100%. Secondary procedures were performed in 8 patients. Stent graft thrombosis led to early conversion (< 30 days) in 3 patients and late conversion (> 30 days) in 3 patients. The stent graft was used as a bridge to open surgery in 2 patients. The overall limb salvage rate was 93%. The mean length of stay was 18.4 days (1-93) and median follow up time 13 months (1-60).

Conclusions: Endovascular repair of traumatic peripheral arterial lesions is a safe and effective treatment in properly selected patients. The rate of secondary procedures emphasizes the need for long term surveillance.

ABSTRACT

Prediction of Essential Language Sites by fMRI as recorded by both Stimulation Mapping and Local Field Potentials

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Sponsored by: Nitin Tandon, MD, Department of Neurosurgery

Supported by: Nitin Tandon, MD, Department of Neurosurgery

Key Words: fMRI, ECoG, CSM, functional mapping

Introduction

Functional MRI (fMRI) is widely used to study language functions in humans. Patients with subdural electrodes (SDEs) provide a unique opportunity to study language in other ways, including cortical stimulation mapping (CSM) and local field potentials (LFPs). There is a paucity of studies that show a comparison of overlap between all three modalities.

Methods

Seventeen patients underwent pre-implantation scanning using a 3T MR. Anatomical images were collected, and functional images were obtained during two visually cued noun and verb generation tasks.

Subdural electrodes (SDEs) were implanted and patients performed the same naming tasks as during fMRI. LFP data were collected. Volumes of interest (VOIs) were generated around each electrode and the response of each one was measured during these language tasks. The t-value of the average mid-gamma (60-120Hz) power within a time window was used to determine the response at each electrode.

All patients underwent CSM in accordance with their clinical needs to identify ESLs during both a visually cued and auditory cued noun generation task. VOIs were generated around each positive electrode to represent the area of depolarization due to current spread along the cortical surface. These VOIs were given intensity values based on the amplitude of the current that caused the deficit.

2562 fMRI data points were chosen that coincided with the VOIs generated from the other CSM and LFP data. Those with significant t-values were determined to be positive fMRI points. These were used to determine overlap with the LFP and CSM data. Any positive fMRI within a given VOI was considered a positive fMRI response in that VOI. Specificity and sensitivity values were calculated.

Results

The results show that when using verb generation fMRI task to predict positive mid-gamma band activity (t-value \geq 11.3) there is 78.12% sensitivity and 69.74% specificity (F1=73.69%). When using the same task to predict ESLs based on a positive CSM result, there is 57.94% sensitivity, and 55.13% specificity (F1=56.5%). Ongoing analysis will evaluate network dynamics during this process.

Conclusion

Different modalities of study allow identification of aspects of underlying networks in language processes. Overlap of these studies can and will give us the most detailed understanding of human language networks possible. An analysis of the data that produces a timeline of activity during fMRI will further allow for the determination of ESLs that exemplify to the “essential versus involved” nature of each individual site. Using these measures allows for surgical resection planning that can avoid these ESLs via a non-invasive and less traumatic procedure for the patient, while also preserving the maximal amount of function.

ABSTRACT

Developing E-Selectin X-Aptamers to Target Ovarian Cancer

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Sponsored by: David G. Gorenstein, PhD, David E. Volk, PhD, Institute of Molecular Medicine

Supported by: Department of Nanomedicine and Biomedical Engineering, and the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Disease

Key Words: Ovarian cancer, X-aptamer, E-selectin

Aptamers are single stranded DNA or RNA oligonucleotides that mimic binding properties of antibodies. The unique sequences conferred by aptamers allow them to take on unique secondary and tertiary structures, yielding various binding specificities due to the specific tertiary shape of the aptamer and the target molecule. The purpose of the project is to develop X-Aptamers that will target the E-Selectin protein found on ovarian cancer endothelial cells. The protein is overexpressed on numerous cancer cells including breast, pancreatic and ovarian cancers. By developing X-Aptamers which can be modified with a variety of ligands, and using them as targeting agents attached to nanoparticles, such as liposomes, we can specifically target chemotherapy to tumor cells, increasing treatment efficacy and lessening side effects. Methods: The library is synthesized on synthetic beads and consists of ~1 million pseudorandom sequences that are designed from known aptamers and have amino (X) linkers in random places. After combining the library with the protein of interest, a magnetic pull down method is used to pick out the sequences that bound to the protein. These sequences are then PCR-amplified and are sent in for NextGen sequencing. After sequencing the aptamer are resynthesized and are tested again (off bead) to ensure the binding was not random. The second step of the project involves using EDC carbodiimide crosslinker chemistry to bind the aptamers, containing a 5'-carboxy group, to a liposome containing a primary amine .. Conclusions: The coupling experiments proved successful when a single stranded 73-mer DNA aptamer containing a carboxy moiety was successfully coupled to a kanamycin molecule which contains primary amino groups. The results were confirmed by observing a shift using gel electrophoresis. The aptamer library selection experiments are still ongoing.

ABSTRACT

Mid-term Outcomes of Endovascular Repair for Patients with Blunt Traumatic Aortic Injury

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Class of 2015

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Supported by: Ali Azizzadeh, MD, Department of Cardiothoracic and Vascular Surgery;
University of Texas at Houston Medical School - Office of the Dean

Key Words:

Abstract: INTRODUCTION: Aortic injury is the second most common cause of death after blunt trauma. Thoracic endovascular aortic repair (TEVAR) has been widely adopted as an alternative to open repair for treatment of traumatic aortic injury (TAI). Although significant short term benefits have been demonstrated for patients undergoing TEVAR, longer term follow up data is lacking. The purpose of this study is to evaluate the mid-term outcomes of TEVAR at an urban trauma center.

METHODS: We analyzed prospectively collected data from the institutional trauma registry. Follow up data was gathered from a combination of medical records, imaging, telephone interview, and Social Security Death Index (SSDI). Primary outcomes were in-hospital mortality, stroke, and paraplegia. Secondary outcomes included device related adverse events (rupture, migration, or endoleak), secondary procedures, open conversion, and all cause mortality.

RESULTS: Between 9/2005 and 7/2012, 82 consecutive patients (57 male, mean age 39.5 +/- 20 years, mean ISS 34 +/- 9.5) underwent TEVAR for treatment of TAI. A total of 87 devices were implanted: TAG (n=36), CTAG (n=12) (WL Gore, Flagstaff, AZ); Talent (n=29), Valiant (n=5) (Medtronic, Santa Rosa, CA); TX2 (n=2) (Cook, Bloomington, IN), and other (n=3). Left subclavian artery coverage was required in 32 (39%) patients. The technical success rate was 100%. The rate of in-hospital mortality, stroke, and paraplegia was 4(5%), 2 (2.4%), and paraplegia 0(0%), respectively. The median follow up time was 2.3 years (range of 0 to 7). The availability of follow up data was: SSDI (100%), telephone interview (68%), clinic visit (64%), and imaging (82%). The incidence of device related adverse events was 2 (2.4%). There were 4 secondary procedures; 2 patients underwent a carotid-subclavian bypass and 2 patients had an open conversion for device related complications. Survival was 95% at 30 days, 88% at 1 year, 87% at two years, and 82% at 5 years.

CONCLUSIONS: At mid-term follow up, TEVAR is an effective and durable option for the treatment of TAI in properly selected patients. Device related adverse events, secondary procedures, and open conversion is rare. Follow up remains a challenge.

ABSTRACT

Allograft treatment of Infected Nonunions of the Forearm

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Sponsored by: Milan K. Sen, MD, Department of Orthopaedics; Mark R. Brinker, MD, Fondren Orthopedics, LLP

Supported by: Volunteer

Key Words: Non-union, allograft, infection, forearm

Infected non-unions of the forearm are a challenging orthopaedic problem. The problem is compounded by loss of bone due to the infection or the initial injury, diseases that impede bone healing such as diabetes and peripheral vascular disease, prior surgical dissection with disruption of vascularity of the bone and the soft tissue envelope, and patient factors such as smoking. The goal of treatment is to eradicate the infection and provide a stable environment for bone healing to occur. Once the infection has been eradicated, bone graft is commonly used to augment healing of defects. Autograft is the gold standard in the treatment of smaller defects. The use of cancellous autograft in larger defects has been less reliable. Corticocancellous autograft has been described for the treatment of larger defects, including either vascularized or non-vascularized fibular grafts, but can result in some morbidity to the patient and involves a second surgical site. Case reports exist using fibular allograft in non-unions of the forearm, but to our knowledge the use of allograft for the treatment of infected non-unions is not common. Moreover the use of radius and/or ulna allograft has not been described. This has the advantage of restoring the anatomic relationship between the bones of the forearm, including the radial bow, allowing for improved forearm motion and function.

The purpose of this project is to determine the outcome of the use of internal fixation with allograft forearm bone in the treatment of infected non-unions of forearm fractures with segmental bone loss. A retrospective review of a single surgeon's patient database was used to identify patients who presented with an infected nonunion of the radius and/or ulna of the forearm. There were 8 patients who qualified for the study from 1998-2012. One of these patients just recently underwent the surgery and so for now is excluded from the study. The success rate of the surgery was determined by the achievement of union at the sight of the allograft implant. In the seven patients that were included, one of them sustained a secondary injury after receiving the allograft repair of his non-union and so his success is unattainable. Out of the remaining 6, 2 patients had to receive secondary surgeries to correct their deformity and were not considered successful. The other four were considered successful. With a current success rate of 66.6% this surgery would be considered a success because the severity of the injury makes attaining a positive outcome very difficult. Another variable to take into consideration is that both of the patients who failed to heal using this procedure were smokers with at least 13 pack years. Smoking has a profound effect on the healing potential of bone and this could have inhibited the efficacy of the allograft procedure. This study will continue with patient surveys to further measure surgery outcome and the most recent patient will be followed to see if his procedure was successful.

ABSTRACT

Tumor Angiogenesis Inhibition by Clodronate in Ovarian Cancer.

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Sponsored by: Anil K. Sood, MD, Department of Gynecologic Oncology, MD Anderson Cancer Center

Supported by: David Volk, PhD, Institute for Molecular Medicine

Key Words: Ovarian Cancer, Angiogenesis, Bisphosphonate

Growth factors such as VEGF are often found at elevated levels in the tumor microenvironment, and induce angiogenesis which promotes tumor growth and expansion. Anti-angiogenic drugs currently being used in conjunction with chemotherapy to treat some cancers target these growth factors or their receptors and can have serious side-effects including stroke and heart attack. Tumor-associated macrophages secrete pro-angiogenic factors contributing to tumor angiogenesis, and correlate with poor prognosis for a variety of cancers. Bisphosphonates, such as clodronate, can selectively inhibit and deplete macrophages, and therefore decrease the concentration of pro-angiogenic factors in the tumor microenvironment. This research focuses on the ability of bisphosphonates to inhibit tumor angiogenesis and may provide an alternative anti-angiogenic treatment with fewer side-effects. An ELISA was performed measuring a variety of pro-angiogenic factors on clodronate treated THP-1 (monocyte), RF-24 (endothelial), and SKOV3 (ovarian tumor) cells, all of which showed a decrease in secreted pro-angiogenic factors of treated cells. Next, a 3D tube formation assay was performed on clodronate treated RF-24 cells, which identified decreased capillary tube formation in treated cells. Then, a migration assay was performed on RF-24 cells with clodronate treatment, VEGF stimulation, or combination to determine the effects of clodronate on endothelial cell recruitment for tumor angiogenesis. Clodronate treated cells had significantly decreased migration ability, which was only partially restored by VEGF treatment. Finally, an in vivo experiment was conducted exploring the effect of clodronate treatment on SKOV3 ovarian cancer model in nude mice. The clodronate treated mice showed significantly decreased tumor growth, increased tumor apoptosis, decreased tumor proliferation, and significantly decreased tumor angiogenesis.

ABSTRACT

Bacterial Contamination of Preservative-free Eye Drops in Single-use Vials of Tafluprost

CHRISTOPHER RICKS

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Sponsored by: Nicholas Bell, MD, Department of Ophthalmology and Visual Science

Supported by: Volunteer

Key Words: ophthalmology, glaucoma, bacterial contamination, tafluprost, preservative-free

Purpose: To investigate the incidence of bacterial contamination of preservative-free tafluprost (ZIOPTAN, Merck & Co., Inc., Whitehouse Station, NJ) after a single exposure to normal eyelid flora.

Patients and Methods: Forty-three eyes were evaluated in this study. Subjects were excluded if they had used topical ophthalmic or systemic antibiotics within the past 4 weeks, preservative-free ophthalmic drops within the past 2 hours, ophthalmic drops with preservatives within the past 12 hours, or ophthalmic ointments within the past 24 hours. Drops of tafluprost were first applied to chocolate and Sabouraud plates. The open tip of the tafluprost vial was then exposed to the participant's eyelashes, and drops from this vial were inoculated onto new plates (Day 0). A separate flock swab elsewhere on the eyelashes was also cultured. Tafluprost from the contaminated vials was also inoculated onto both types of plates 1 day and 6 days later. Using standard microbiological techniques, the plates were incubated and evaluated. Frequency and speciation of microbial growth on contaminated plates was recorded. A stepwise logistic regression was used to identify risk factors associated with microbial contamination.

Results: Of the 43 subjects, 35 (81%) flock swabs obtained from 43 subjects showed bacterial growth. Vials from 2 subjects showed pre-contamination, and those plates were discarded. Of the 41 remaining vials, 53.7% (22 vials) showed growth on Day 0. After 24 hours (Day 1), 14.6% (6 vials) exhibited growth. After 144 hours (Day 6), 7.3% (3 vials) exhibited growth. A total of 8 types of bacteria were cultured; the most common were coagulase-negative *Staphylococcus* species (82.7%) and *Staphylococcus aureus* (6.5%). There were no risk factors identified for growing bacteria.

Discussion and Conclusion: Twenty-four hours after contamination by eyelashes, cultures of preservative-free tafluprost demonstrated bacterial growth in 14.6% of samples. At 6 days, only 7.3% had bacterial growth. A limitation of this study is that the vials were not re-exposed to eyelids/eyelashes every day, as may occur with multiple dose administrations, and hence we cannot extrapolate to what happens after multiple re-exposures.

ABSTRACT

Hypothermia Effects on Thrombolytic Activity of Tissue Plasminogen Activator in Acute Ischemic Stroke Treatment

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Sponsored by: James Grotta, MD, Department of Neurology

Supported by: James Grotta, MD, Department of Neurology

Key Words: Thromboelastography, hypothermia, tissue plasminogen activator (tPA), stroke

Background: Acute ischemic stroke affects over 400,000 patients in the United States each year. Tissue plasminogen activator (tPA) is currently the only approved treatment for these patients, but other concurrent studies such as ICTuS (intravascular cooling in the treatment of stroke) suggest that supplementing hypothermia treatment provides a neuroprotective function and can improve clinical outcome. As an enzyme, the thrombolytic abilities of tPA may be subject to varied effectiveness in the cooler environment produced by hypothermia treatment. Thromboelastography (TEG) is a technology which quantifies the speed and strength of clot formation and lysis using whole blood, helpful in establishing a unique “clotting profile” for each ischemic stroke patient. We hypothesize that one or more TEG parameters will demonstrate diminished clot-busting abilities by tPA due to hypothermic conditions.

Methods: Acute ischemic stroke patients receiving tPA within 4.5 hours of symptom onset were included. Blood was drawn prior to and 10 minutes after tPA bolus. Both ‘baseline’ and ‘post-tPA’ blood samples were split into three groups for analysis by the TEG machines at 37°C, 33°C, and 30°C. The 33°C and 30°C samples were cooled in temperature-controlled baths for 20 minutes prior to TEG analysis to simulate hypothermia treatment. Baseline TEG data at all three temperatures was also obtained from 10 control patients of similar stroke patient age to account for the effects of hypothermia on control blood.

Results: In control patients, Angle averages were 45.5, 51.5, and 60.9 ($p=0.041$, $N=7$) and Delta averages were 1.4, 0.9, and 0.7 ($p=0.038$, $N=7$) for the 30°C, 33°C, and 37°C blood samples respectively. Ischemic patient K-values averages were 2.4, 2.0, and 1.6 ($p=0.018$, $N=8$) at the same temperatures. Also at the same temperatures, post-tPA ischemic LY30 averages were 94.7, 94.5, 94.8 ($p=0.320$, $N=8$).

Conclusions: As demonstrated by the Angle (rate of clot formation), Delta (time to form initial 2mm clot), and K-value (time from 2mm to 20mm clot growth) averages, decreasing temperature causes progressively slower clot formation. Also, the fibrinolytic ability of tPA (as was measured by the LY30 value) remained relatively constant as temperatures decreased. Hypothermia proved to slow clot formation, but demonstrated no change in the lysing ability of the tPA enzyme.

ABSTRACT

Intravenous Autologous Bone Marrow Mononuclear Cells May Enhance Recovery of the Corticospinal Tract in Ischemic Stroke

CORY G. ROBINSON *The University of Texas at Houston Medical School* *Class of 2015*

Sponsored by: Sean Savitz, MD, Department of Neurology

Supported by: Sean Savitz, MD, Department of Neurology; University of Texas at Houston Medical School – Office of the Dean

Key Words: Stem cells, ischemic stroke, corticospinal tracts, DTI

Background and Purpose: Wallerian degeneration of the corticospinal tract (CST) after ischemic stroke (IS) can be characterized on MRI by diffusion tensor imaging (DTI). DTI has not been used yet as a biomarker to test investigational neurorestorative therapies for patients with IS. In our ongoing clinical trial investigating the safety of autologous bone marrow mononuclear cell infusion in patients with IS, we aimed to use DTI to explore the potential effects of a cell therapy on the recovery of the CST.

Methods and Materials: As part of a clinical trial, 16 IS patients underwent bone marrow harvest and intravenous infusion of autologous mononuclear cells. In another comparable cohort, 10 IS patients were also prospectively studied but did not receive bone marrow mononuclear cells. In all patients, multimodal MRI which included DTI was performed at various times after stroke to assess structural changes within the brain. Using Analyze 10 software modules, FA maps were generated and analyzed by drawing ROIs around the cerebral peduncles. The mean of three independent measurements were used to interpret the results. The relative FA (rFA) of the CST in the cerebral peduncle was measured for the ipsilateral side compared to the contralateral side, indicating the level of damage or Wallerian degeneration to the CSTs.

Results: In the group treated with bone marrow cells, the mean rFA (0.70) was significantly higher at day 90 compared to the non-treated group (0.57).

Conclusions: This study suggests that autologous bone marrow derived cells may enhance recovery of the CST. These results are limited by their preliminary nature and small sample size. In addition, the data were derived from a comparison of two independently obtained groups.

ABSTRACT

Effect of intravenous anti-IL6 on inhibitor formation and spleen germinal center development in haemophiliac mice

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Sponsored by: Keri Smith, PhD, Department of Pathology and Laboratory Medicine.

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: IL6, anti-IL6, haemophilia, inhibitor antibodies

Background: Haemophilia A (HA) is characterized by a deficiency in functional Clotting Factor VIII (FVIII). Treatment of the disease with intravenous injections of recombinant human FVIII (hFVIII) is often accompanied by a pathological immune response that results in the production of functionally inhibitory anti-FVIII antibodies. Inflammation and tissue damage is associated with inhibitor production. We focused our study on IL6, an inflammatory cytokine associated with the capacity to drive antibody secretion. We hypothesized that treatment of haemophiliac mice with anti-IL6 monoclonal antibody in conjunction with hFVIII therapy would reduce levels of circulating inhibitor antibodies. We also hypothesized that treatment with anti-IL6 would inhibit germinal center development in the spleen. **Methods:** Mice were treated with either 2.0ug of hFVIII or isotype antibody once a week for four weeks. Relative levels of inhibitor in mice plasma were measured by FVIII ELISA. Mice spleens were extracted and cultured for characterization via FACS before and after CD4-enrichment. To elaborate on information obtained from FACS, an ELISPOT was performed on a population of splenocytes cultured with anti-IL6 for five days following a CD138 depletion. **Results:** When treated with anti-IL6, the average titer was reduced from 1:555 to 1:122. FACS analysis of splenocytes from these mice show increased percentage of CD25⁺ cells (38.9% gated vs. 6.34%, $p < 0.02$). This was not accompanied by increased expression of intracellular FoxP3. Curiously, treatment with anti-IL6 also resulted in increased percentage gated of B-cell marker B220⁺CD19⁺ splenocytes (41.6% vs 26.5%, $p < 0.03$). ELISPOT showed that anti-IL6 treatment decreased the average number of anti-FVIII specific spot-forming cells from 13.9 to 5.0 per million ($p < 0.01$) but increased total IgG producing cells from 6300 to 15420 per million ($p < 0.01$). **Discussion:** Anti-IL6 reduces production of inhibitor antibodies in haemophiliac mice, and this reduction appears to occur by a T_{reg} independent pathway. The reduction also appears to occur by a mechanism more specific than simply global mediation of immune response.

ABSTRACT

Timing of fresh frozen plasma transfusion for warfarin reversal in traumatically injured patients: a retrospective cohort study

JAIMIN SHAH

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Sponsored by: James McCarthy, MD, and Amy Noland, MD, Department of Emergency Medicine; John Holcombe, MD, and Charlie Wade, MD, Department of Surgery

Supported by: James McCarthy, MD, Department of Emergency Medicine

Key Words: Warfarin, Trauma, International Normalized Ratio, Fresh Frozen plasma

Introduction: Patients at risk for thromboembolic events require systemic anticoagulation. Warfarin has been a reliable anticoagulant and the mainstay for outpatient therapy for many years. However, patients with therapy are at risk for bleeding complications especially with minor injury. Bleeding complications are the major cause of death for traumatically injured patients in the first 24 hours. Coagulopathy is common in the bleeding patient and is compounded by pre-injury anticoagulant use. Fresh frozen plasma (FFP) has been the mainstay of emergent reversal of the warfarin induced coagulopathy in the bleeding injured patient for many years. Effective hemostasis and reversal of coagulopathy is crucial in limiting complications in trauma patients.

In an effort to improve the speed of availability of FFP, in 2/2010 the Memorial Hermann emergency department (ED) began storing thawed FFP in the ED to minimize delays in administration. We believe that by having accessible FFP, time to treatment, time to normalize coagulopathy and mortality would all decrease.

Our hypothesis is that patients with traumatic injuries who are coagulopathic from prophylactic warfarin and need rapid reversal, there will be a difference in timing of FFP administration. We will evaluate the timing of administration before and after the change in availability of FFP in 2010.

Purpose: The specific aim of our project was to evaluate the effect of storing FFP in the ED on order timing and product administration in traumatically injured patients who are on pre-injury prophylactic warfarin therapy.

Methods: We queried the Trauma Database for all patients taking pre-injury warfarin from 1/1/2008-12/31/2011. We evaluated the timing difference of: "arrival to FFP administration" pre and post 02/01/2010, Glasgow Coma Scale (GCS), and mortality were evaluated. Patients who received FFP > 4 hours after arrival were judged non-emergent and excluded. We used

two tailed t-tests and chi square to analyze our data set. Patients receiving plasma prior to ED admission or directly admitted to another inpatient unit were excluded.

Results: Of 230 patients taking warfarin 82 (35%) received FFP in ED (32 pre and 50 post intervention), and 116 patients (50.1%) did not, 14% received FFP after transferring to an inpatient unit. There was no difference in mortality before FFP could be administered (6.52% vs. 6.17% = 0.2). In treated patient there was no difference in initial INR (median 2.43 vs. 2.37 P=0.32). Initial INR was lower in untreated vs. treated patients (2.26 vs. 3.26 P=0.007). ISS was significantly higher in the treated vs. untreated (17.0 vs. 12.18 P=0.0001). When FFP was available it was administered earlier (114.76±52.7 vs. 84.46±60.13 min P 0.0476). There was no difference in mortality pre and post intervention (17% vs. 20%). There was no significant difference in FFP administration time when groups and GCS<15, GCS<11, ISS>16, or ISS>25 were compared.

Conclusion: For patients requiring emergent treatment with FFP, the storage of thawed FFP in the ED significantly decreased the time to administration. For patient taking pre-injury warfarin ISS and INR were the apparent major drivers in decision to administer FFP. Given the retrospective nature of this analysis, timing of reversal of coagulopathy could not be reliably determined as there was no standard timing of lab draws. While the increased availability of FFP shortened time to treatment, further analysis will be needed to determine if it improves mortality and other patient centric outcomes.

ABSTRACT

The Epidemiology of Sepsis in Critically Injured Trauma Patients

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Sponsored by: Laura J. Moore, MD, Department of Surgery

Supported by: Laura J. Moore, MD, Department of Surgery, Department of Translational Injury Research (CeTIR)

Key Words: Sepsis, trauma, patients, epidemiology

The purpose of this study is to analyze the current epidemiology of sepsis in critically injured trauma patients at our urban level 1 trauma center. A retrospective cohort study was performed looking at all adult trauma patients admitted to the Shock Trauma Intensive Care Unit (STICU) at Memorial Hermann Hospital from 1/1/2009 to 12/25/2010. Data was collected on patient demographics, injury severity score (ISS), hospital length of stay (LOS), STICU LOS, presence of sepsis, severity of sepsis, source of infection, development of organ failure, discharge disposition, and mortality. The presence of organ and renal failure were determined by coding in the Trauma Registry data used in the analysis. Patients were then grouped into the categories of no sepsis (n = 298) and sepsis (n = 167). The two groups were then compared using the Student's t test. A p value of <0.05 was considered significant.

There is an association between sepsis and increased length of hospital stay (p-value < .0001) and increase in ICU length of stay (p < .0001). With respect to outcomes, the data showed an association between sepsis and development of organ failure (p = .0003). In terms of discharge disposition, patients without sepsis were more likely to be discharged home as compared to patients with sepsis (p=0.006). Progression to severe sepsis also shows an increase in mortality (p = .0030, <.0001), hospital length of stay (p = .0116), ICU length of stay (p = .0047), organ failure (p < .0001), and renal failure (p < .0001) These findings indicate that the development of sepsis in critically injured trauma patients is a significant contributor to hospital cost and patient morbidity and mortality.

ABSTRACT

Differences in Social Interactions in 4 and 6-Month-Old Infants Based on Later Autistic Spectrum Disorder (ASD) Classification

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Supported by: Pauline A. Filipek, MD, Department of Pediatrics – Children’s Learning Institute and the Division of Child and Adolescent Neurology; University of Texas at Houston Medical School – Office of the Dean

Key Words: ASD, autism, infants, social interactions

Introduction

Autistic spectrum disorders (ASDs) include deficits in social interactions, affecting verbal and non-verbal communication that emerge before six months of age. Current screening instruments do not permit identification of signs of ASD before 9-12 months of age and the average age at the time of diagnosis continues to be age ~5 years. ASD prevalence has been steadily increasing in recent years. Early behavioral intervention could greatly ameliorate the prognosis of affected individuals by reducing or even eliminating the developmental impairment and need for assistance.

Methods

In this retrospective, double-blinded, observational study 47 videotapes of infants, recorded with a fiberoptic eyeglass camera at four and six months of age, were analyzed for social behaviors during interactions with a parent and investigator. Some of the enrolled subjects were siblings of a child with established ASD diagnosis (SIBs). The cohort was divided into ASD (n=17) and Non-Spectrum (N/S; n=30) groups based on ADOS-Toddler module applied at 12-18 months. The coders were blinded to the diagnosis. Eye contact, smiles, crying and vocalizations were coded using Noldus Observer™ 9.0 and comparatively analyzed for rate, frequency, mean and total duration, and total number. Statistical analysis using the Mann-Whitney U test was used to compare the variables across ASD and N/S groups.

Results

For the infant-parent paradigm, 4-month-old ASD infants displayed lower frequency ($p<0.05$) of undirected smiles and undirected vocalizations during Pre-still Face and Still Face. A similar trend was observed in the 6-month-old ASD group, with significant findings ($p<0.05$) in eye contact, total directed and directed positive vocalizations, and directed smiles in Pre- and Still Face.

For the infant-investigator paradigm, in 4-month-old infants, significant differences ($p<0.05$) were found for undirected positive, negative, and total vocalizations. The ASD group displayed greater activity than the N/S group in these variables during Pre- and Still Face. No significant differences were found in the 6-month-old group.

Conclusion

These results confirm the hypothesis that 'red flags' for ASD can be identified in infants younger than six months of age; larger cohort studies are essential in order to establish reliable criteria for earlier diagnosis of ASD.

ABSTRACT

Grem1 Deletion Reduces Susceptibility to Chronic Pancreatitis

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Sponsored by: Tien C. Ko, MD, Yanna Cao, MD, Department of Surgery

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: Gremlin, Pancreatitis, Bone Morphogenetic Protein

INTRODUCTION: Bone morphogenetic proteins (BMPs), members of the transforming growth factor (TGF)- β superfamily, have an anti-fibrogenic function in the kidney, lung, and liver. We recently demonstrated that BMPs have an anti-fibrogenic role in the pancreas and that BMP type II receptor knockout in mice had increased susceptibility to chronic pancreatitis (CP) induction. Gremlin, a natural BMP antagonist, is elevated in the fibrotic lung and kidney, suggesting a pro-fibrogenic role of gremlin in these diseased organs. We hypothesize that during CP progression gremlin is increased and promotes fibrosis by antagonizing the anti-fibrogenic BMP signaling. **METHODS:** CP was induced by intraperitoneal injections of cerulein (50 $\mu\text{g}/\text{kg}$, 5 hourly injections, 3 days a week) for up to 8 weeks in Swiss Webster mice (8-12 weeks old); control mice received normal saline injections. Heterozygous gremlin knockout mice ($Grem1^{+/-}$) and wildtype control littermates ($Grem1^{+/+}$) underwent the same CP induction with cerulein for 4 weeks. Pancreatic fibrosis was assessed by Sirius Red staining for collagen deposition, and gremlin expression was evaluated by immunohistochemistry on paraffin sections. Gremlin expression was assessed by immunohistochemistry on paraffin sections of human normal and CP pancreata. **RESULTS:** In Swiss Webster mice, collagen deposition showed a time-dependent increase at 4 (5.4-fold) and 8 weeks (10.7-fold) in CP mice versus control mice ($p < 0.01$); a time dependent increase in gremlin expression was observed at 1, 2, 4, and 8 weeks induction (7.0-fold; 44.7-fold; 123.7-fold; 48.4-fold) in CP mice versus control mice ($p < 0.001$). Gremlin expression was localized to pancreatic stellate cells and duct like cells in mouse CP pancreata. Furthermore, gremlin was increased in human CP pancreata compared to normal subjects. In $Grem1^{+/-}$ knockout mice, collagen deposition was reduced (30%) compared to $Grem1^{+/+}$ wildtype littermates under CP induction ($p = 0.028$). **CONCLUSIONS:** Increased gremlin expression in both mouse and human CP pancreata validates the mouse CP model as a clinically relevant model for the study of gremlin's role in CP progression. In the mouse CP model, gremlin expression increased prior to and along with fibrosis development. Furthermore, gremlin knockout mice showed reduced fibrosis compared to wildtype mice following CP induction. Our results in this study suggest that increased gremlin promotes pancreatic fibrosis during CP progression. The effects of increased gremlin on anti-fibrogenic BMP signaling warrant further investigation.

ABSTRACT

Targeting calreticulin as a biomarker for ⁶⁴Cu PET imaging of early-stage atherosclerosis

TAYLOR SUGG

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Class of 2015

Sponsored by: Patrick Kee MD, PhD, Delia Danila PhD, Department of Internal Medicine

Supported by: Patrick Kee MD, PhD, Department of Internal Medicine; University of Texas at Houston Medical School – Office of the Dean

Key Words: calreticulin, atherosclerosis, PET

Background Several protein chaperones play a key role in the unfolded protein (UPR) or cell stress response seen in various stages of atherosclerosis. In particular, calreticulin, a 46-kDa lectin involved in endoplasmic reticulum Ca²⁺ homeostasis and protein folding, is selectively expressed on the cell surface during the immunogenic cell death observed in early-stage plaque formation. Our **objective** is to develop a non-invasive imaging technique to visualize inflammatory atheroma with Positron Emission Tomography (PET) tracers targeting increased plaque-associated calreticulin expression. We developed a two-pronged approach for the tracer synthesis, using both anti-calreticulin antibody and a commercially synthesized calreticulin-targeting peptide. The peptide was pre-synthesized with rhodamine-B while the antibody was conjugated to 5-aminofluorescein using sulfo-NHS and EDC peptide conjugation. Rhodamine-B and 5-aminofluorescein are both fluorophores used to confirm tracer presence in aortic tissue through histological detection. Both of these molecules were then conjugated to the chelator, CB-TE2A, for Cu-64 radiolabeling. The success of the conjugation of the tracers to CB-TE2A was confirmed using a size-exclusion Sephadex-25 column, high performance liquid chromatography (HPLC) and spectrophotometry. Binding affinity for both the antibody and peptide conjugates for calreticulin was tested using a modified ELISA test with a horseradish peroxidase secondary antibody. In-vitro binding affinity has demonstrated 3-4-fold increase in measured absorbance for the antibody conjugate and a 1-2 fold increase for the peptide.

ABSTRACT

Use of Thromboelastography as a predictor of sepsis in trauma patients

WILLIAM TYSON

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Class of 2015

Sponsored by: Laura J. Moore, MD, Department of Surgery

Supported by: Laura J. Moore, MD, Department of Surgery

Key Words: Sepsis, TEG, Thromboelastography, Trauma

TEG provides an overall view of ex vivo coagulation in patients. Hyper- and hypocoagulability, as demonstrated with TEG, has been shown to correlate with clinically relevant morbidity and mortality. Septic shock is the tenth leading cause of death in the United States, the leading cause of death in non-cardiac intensive care units (ICUs), and is the leading cause of death in surgical patients. Studies indicate that early identification of patients at risk for sepsis and early administration of targeted therapies improves clinical outcomes in patients. This study was a retrospective analysis of a prospectively collected database of serial TEG values in adult trauma patients between December 2010 and January 2012. Patient sepsis status was assessed and added to an already created TEG database of 1125 patients who had TEG values recorded. Each patient was assessed to determine if they had sepsis based on the ACCP/SCCM sepsis definitions. In addition, sepsis severity, source of infection, and Denver multiple organ failure (MOF) score were documented. Patients were divided into two groups; those that developed surgical sepsis and those that did not. Admission TEG values were then compared between the two groups. We then compared admission TEG values in sepsis patients with sepsis progression. The same analysis was done on patients with infection, pneumonia and SIRS. Assessment of the average TEG associated values (MA, K, R, LY30, ACT, Alpha) for the sepsis and non-sepsis groups indicated that patients in the sepsis group had statistically different ACT ($P = 0.01$), Alpha ($P = 0.002$), R ($P = 0.03$), & K ($P = 0.001$) values than the non-septic group. However, in the ACT and R values this was most likely due to the effect of outliers, as indicated in the graphs to the left. In patients with sepsis, K-values were elevated, 1.6 to 1.3 respectively, and Alpha was slightly lower than in patients without sepsis (71 to 74 respectively). No statistically significant correlations were seen with admission TEG values and sepsis progression or SIRS. Statistically significant differences in patients with and without infection were observed in K (1.5 and 1.3 respectively, $p = .02$) and Alpha (72 and 74 respectively, $p = .04$). Statistically significant differences in patients with and without Pneumonia were observed in Alpha (72 to 74 respectively, $p = .04$). Based on the results of our study, we conclude that patients with sepsis have an increased K-Time and lower alpha values on admission TEG as compared to patients without sepsis. Increased K time and lower alpha values in septic patients compared to non-septic patients indicated that these values could be used as an indicator of risk for sepsis. No admission TEG values were able to predict sepsis progression, most likely due to the increased time period between admission TEG and diagnosis of sepsis progression. TEG values Alpha and K-time were seen to be predictive of general infection and could be used to initiate protocols to prevent infection. Lastly, it was noted that Alpha holds predictive value in patients susceptible to pneumonia, so it could be used in assessing pneumonia risk in patients.

ABSTRACT

Pharmacological Interventions to Overcome Reactive Oxygen Species in Hemorrhagic Shock

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Sponsored by: Marie-Francoise Doursout, PhD, Department of Anesthesiology

Supported by: Marie-Francoise Doursout, PhD, Department of Anesthesiology; University of Texas at Houston Medical School – Office of the Dean

Key Words: Hemorrhagic shock (HS), reactive oxygen species (ROS), nitric oxide (NO)

Background

In previous studies it has been shown that hemorrhagic shock (HS) leads to the production of reactive oxygen species (ROS). This includes the production of nitric oxide (NO) due to the activation of inducible nitric oxide synthase (iNOS) during hemorrhage. The ROS produced cause lung injury and vascular endothelium injury, which causes endothelial dysfunction and leads to multiple organ failure. NO specifically is a vasodilator that decreases blood pressure due to its activation of the enzyme soluble guanylyl cyclase (sGC), leading to the formation of cGMP, which ultimately causes smooth muscle relaxation and vasodilation. It is also known that NO reacts with superoxide anion to form peroxynitrite, a powerful oxidant that damages organ tissues.

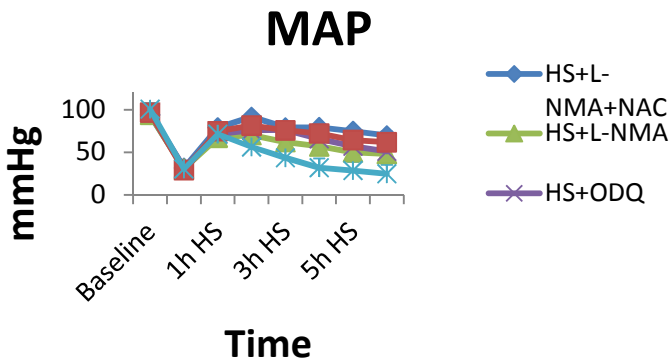
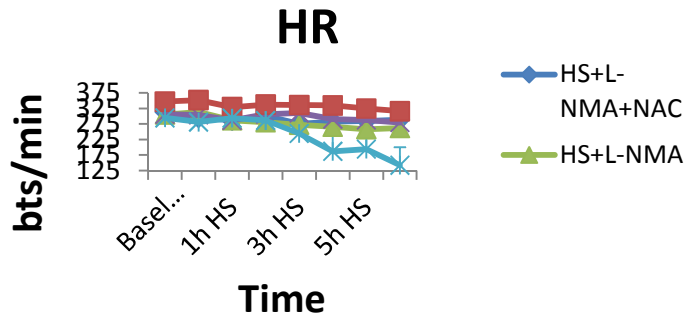
Purpose

The purpose of this study was to identify the effectiveness of different pharmacological interventions in overcoming ROS induced hypotension and lung injury in rats subjected to hemorrhagic shock.

Methods

Male Sprague-Dawley rats were used and groups of 4 rats were tested for each pharmacological intervention. The drugs tested were NG-methyl-L-Arginine (L-NMA) a NOS inhibitor, N-acetylcysteine (NAC) a conventional ROS inhibitor, ODQ a sGC specific guanylyl cyclase inhibitor. The study consisted of 5 groups: control (HS with no pharmacological intervention), HS+L-NMA, HS+L-NMA+NAC, HS+ODQ, HS+ODQ+NAC. The rats were anesthetized, catheterized, and then blood was drawn via catheter until the blood pressure reached 30 mmHg in order to subject them to hemorrhagic shock. The rats were then monitored for 6 hours, with blood samples being taken at baseline, end of HS, 2 hours, 4 hours and 6 hours in order to test for NO and cytokine production. At 1 hour post HS a drug or drug combination under investigation was given. At the end of the experiment lung samples were taken for wet-to-dry, immunochemistry and pathology studies.

Results



Conclusion

While all of the inhibitors (administered alone) are likely to improve heart rate and mean arterial blood pressure compared to HS alone, our data shows that when combined with NAC, an ROS inhibitor showed the greatest recovery as the HS induced decrease in MAP and HR in NAC groups were almost restored to baseline values. We are still waiting on the results from the immunochemistry and pathology labs on the lung samples to determine the amount of inflammation found in each group.

ABSTRACT

Activation of endothelial Nitric Oxide Synthase by hydro-alcoholic plant extract induces vascular relaxation of rat aortic rings.

KAI M. WANG

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Sponsored by: Iraida G Sharina, PhD, Department of Cardiology

Supported by: Iraida G Sharina, PhD, Department of Cardiology; University of Texas at Houston Medical School – Office of the Dean

Key Words: Vasodilation, Nitric Oxide, Hypertension

Treatments modulating the NO/cGMP signaling pathway (nitrovasodilators) are widely used to manage cardiovascular disease. However, they are associated with various negative side effects such as nitrate tolerance and tissue damage by nitration of cellular proteins and lipids. Therefore, there is an urgent need for the development of alternative therapeutics to improve vascular health and function. In this work, we investigated effects of several plant extracts used in traditional medicine to alleviate symptoms related to hypertension.

We tested several hydro-alcoholic extracts for their ability to induce dose-dependent relaxation in explanted rat aortic rings (AWC-11-071). The rings were prepared from the descending thoracic aorta of freshly sacrificed rats, mounted in specialized organ chambers to record the changes in the isometric tension by a force transducer and pre-contracted with 100nM phenylephrine (PE). We identified the leaf extract BAL as the most potent inducer of dose-dependent relation; 0.05 mg/ml of extract elicited full relaxation of aorta. We performed preliminary biochemical characterization of the active ingredient in this extract. Using size exclusion filtration we demonstrated the active fraction has an approximate MW of 10-30kDa, counter-indicative of small molecular weight nitrogen-containing or polyphenol compounds demonstrated previously to induce vasorelaxation. Activity of the extract was not soluble in organic solvents (hexane). It was also relatively heat stabile, as its vasorelaxative potency was decreased by 46% upon heating for 10 minutes at 95°C. Using pharmacological dissection approach, we attempted to identify the molecular target for the extract's activity. We sequentially tested various inhibitors for endothelial nitric oxide synthase (eNOS), soluble guanylyl cyclase (sGC) and Protein Kinase G (PKGI α). Our results demonstrated that the BAL extract induces potent vasorelaxation in endothelium intact but not endothelium-denuded aortic rings treated with PE. Inhibitors of eNOS (100 μ M L-NAME; 1.5 μ M SEITU), sGC (30 μ M NS2028), and PKGI α (30 μ M Rp-8-Br-cGMPS, 8 μ M PKGI α Inhibitor) were able to completely inhibit the relaxation induced by BAL.

Our results indicate that our extract exerts its activity through the activation of the NO/cGMP signaling pathway and identify this plant as potential source of therapeutics for the management of blood pressure.

ABSTRACT

Outcomes Research: Mandibular Fractures in the Diabetic Population

NORMAN H. WARD III The University of Texas at Houston Medical School Class of 2015

Sponsored by: David Wainwright, MD, Department of Surgery

Supported by: David Wainwright, MD, Department of Surgery

Key Words: Mandible, Diabetes, Fracture, Complications

Objective: To record the incidence of complications following mandibular fracture repair in diabetic patients presenting to Memorial Hermann Hospital from 2007 to 2011.

Diabetes mellitus has become an increasingly prevalent disease process in the United States, resulting in poor wound healing and increasing the incidence of post-surgical complications. Considering their high vulnerability to morbidities after surgery, limited study has been conducted on the specific complications following mandibular fracture repair focusing on the diabetic population. A retrospective chart review of 35 patients with mandibular fractures and preexisting diabetes mellitus presenting to Memorial Hermann Hospital TMC between 2007 and 2011 was conducted to analyze specific complication types and frequencies. Complications occurred in 17 (49%) patients with 64 total fracture sites. Out of 35 patients, the most common complications were various forms of infection, 7 (20%), and nerve injury, 7 (20%), followed by malocclusion 5 (14.3%), wound dehiscence/hardware exposure 4 (11.4%), and to a lesser degree fistula, trismus, and mal-union 2 (5.7%), 2 (5.7%) and 1 (2.9%) respectively. Length of stay, time between injury and repair, the presence of additional facial fractures and co-injuries, mechanism of injury, history of hypertension and/or hyperlipidemia, surgical approach, fixation method, fracture locations, and glucose maintenance were not statistically different in patients with and without post-surgical complication ($p > 0.05$). When comparing these rates to the available literature, the overall incidence of complications seems to be greater in this subpopulation, although future comparison to a control population would be preferred.

ABSTRACT

The Effects of Testosterone Deficiency on Fracture Healing

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Sponsored by: Catherine G. Ambrose, PhD, Department of Orthopedic Surgery

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: Fracture healing, testosterone, hypogonadism

It has been estimated that as many as 84% of fracture patients that fail to produce a solid bony union after proper reduction and stabilization (non-union) can be diagnosed with one or more endocrine or metabolic disorders. It is our hypothesis that hypogonadism, specifically low testosterone, will lead to impaired fracture healing.

Two groups of 8 male Sprague Dawley rats were compared in their rate and quality of fracture healing: a control group of intact rats, and a castrated group with low testosterone. Fractures were surgically induced at the middle third of the femur using nail clippers. The fracture was stabilized with K-wire pin fixation and the animals were given time to heal. After sacrifice and imaging at 2 and 4 weeks after surgery, the femurs were removed and subjected to mechanical testing. Blood tests confirmed testosterone deficiency in the castrated animals.

There was no significant difference in percent healing between the castrated and intact animals at either the 2 week or the 4 week timepoints. The mechanical strength of the femurs (both fractured and control) was lower in the castrated animals compared to the intact animals at both 2 and 4 weeks, but none of the differences were significant. As is seen in estrogen-deficient osteoporosis, lumbar BMD in castrated specimens was shown to be significantly decreased at 4 weeks compared to the intact group ($p < 0.05$). Unlike models of estrogen-deficient osteoporosis, this model of hormone deficiency demonstrated no significant differences in femoral neck strength with respect to time or testosterone levels. This evidence suggests that within a 4 week window of healing, there is no apparent detrimental effect of testosterone deficiency on fracture healing.

ABSTRACT

Improving the effectiveness of tobramycin-loaded polymer microspheres in the treatment of osteomyelitis

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Sponsored by: Catherine G. Ambrose, PhD, Department of Orthopedic Surgery

Supported by: National Institute of Diabetes and Digestive and Kidney Diseases,
5T35DK7676-20

Key Words: microspheres, microparticles, biodegradable, polymer, osteomyelitis

Often times, following orthopedic surgery, patients present with additional problems, one of these being infection of the damaged bone, termed osteomyelitis. Currently, osteomyelitis is treated by long-term intravenous antibiotics and surgical debridement of infected necrotic bone (Ross 2). Because of poor penetration of the antibiotic, particularly tobramycin, high serum concentrations need to be used for extended periods, which can be associated with nephrotoxicity, ototoxicity and gastrointestinal side effects. Microspheres have proven useful as a localized, biodegradable drug-delivery system, and were used in a recent study as a drug-delivery method for the delivery of tobramycin to treat osteomyelitis (Ambrose 1). Also, it has been suggested that Vascular Endothelial Growth Factor (VEGF) stimulates angiogenesis and that by promoting angiogenesis in the area of bone damage and osteomyelitis, both the stimulation of bone healing and the treatment of the infection, may be accomplished (Ross 2). We hypothesize that both tobramycin and VEGF can be physically combined into a single gelatin-coated microsphere structure, without altering or reducing the effectiveness of one another, and that this structure can still degrade and release these agents in a controlled and desirably timed manner. In our study, we will manufacture a treatment system composed of a biodegradable poly(lactic-co-glycolic acid) (PLGA) microsphere core, loaded with the antibiotic tobramycin, and encapsulated within a biodegradable vascular endothelial growth factor (VEGF)-loaded gelatin capsule. The core of our microspheres will be formed using a double emulsion-solvent extraction technique (Ambrose 1). We will coat our particles by soaking them in a gelatin solution. Following this encapsulation, we will crosslink the outer gelatin coat, and then soak the cross-linked, coated spheres in a VEGF solution, to allow for diffuse-loading of VEGF. We will characterize our coated microspheres by evaluating the efficiency of the gelatin coating, the loading efficiency and elution rate of tobramycin and VEGF, particle morphology, size, and surface charge. All of these characteristics can be altered by adjusting the timing of particular steps of our experiments, the cross-linking method used to crosslink the outer gelatin coating, the concentrations of PLGA and Tobramycin in the microsphere core, and/or the concentrations of gelatin and VEGF in the outer coating. These properties can be adjusted until we achieve microspheres with the characteristic dissolution and drug eluting timelines that are necessary to effectively treat the targeted infection.

ABSTRACT

The Effects of Sanguinarine and Rolipram on Serotonin-Induced Long-Term Excitability in *Aplysia* Sensory Neurons

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Sponsored by: John H. Byrne, PhD, Department of Neurobiology & Anatomy

Supported by: National Institute of Neurological Disorders and Stroke, 5 T35 NS 64931-3

Key Words: Sanguinarine, Rolipram, LTE, *Aplysia*, PDE, MKP-1

Learning and memory have long been studied at the synaptic level. Recently more emphasis has been placed on the nonsynaptic mechanisms of learning and memory. One of the measures of this is long-term excitability (LTE). LTE is defined as a change in the responsiveness (e.g., number of elicited action potentials) of a neuron in response to a constant stimulus. Sensitization, a simple form of memory, of withdrawal reflexes in *Aplysia californica* is associated with a long-term (24 h) change in excitability of sensory neurons (SNs) involved in the withdrawal reflex. Application of the neurotransmitter serotonin (5-HT) to isolated SNs in culture is known to mimic these effects of sensitization training.

One of the molecular pathways involved in 5-HT-induced memory is the extracellular signal-regulated kinase (ERK) biochemical cascade. LTE has been shown to be modulated by ERK, a mitogen-activated protein kinase (MAPK) isoform, via cAMP response element-binding protein 2 (CREB2) phosphorylation. CREB2 is a gene transcription repressor of long-term effectors of cell excitability which is inactivated when it is phosphorylated. Sanguinarine increases phosphorylated ERK (pERK) levels, the active form of the kinase, as it is a specific inhibitor of MKP-1, a dual specificity phosphatase (DUSP). We hypothesized that Sanguinarine would enhance the effects of 5-HT on LTE. Unfortunately, at concentrations that Sanguinarine had been shown to increase pERK it had a toxic effect on cultured SNs.

A parallel pathway to the ERK pathway that also modifies 5-HT-induced memory is mediated by protein kinase A (PKA). PKA is activated when intracellular cAMP levels rise. PKA then phosphorylates cAMP response element-binding protein 1 (CREB1). CREB1 is a transcription factor for genes that enhance long term memory markers. Rolipram is a phosphodiesterase (PDE) inhibitor that has been shown to increase cAMP levels. Moving forward, we hypothesize that Rolipram could be an enhancer of 5-HT's memory effects. Preliminary data is promising, suggesting that Rolipram may more than double the LTE increases seen when using 5-HT treatments alone. However, more work needs to be done in order to clarify Rolipram's effects.

ABSTRACT

Spatial Learning and Cavity Volume Analysis following Multipotent Adult Progenitor Cell Therapy in Traumatic Brain Injury

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Sponsored by: Charles S. Cox Jr., MD, Department of Pediatric Surgery

Supported by: Charles S. Cox Jr., MD, Department of Pediatric Surgery

Key Words: TBI, MAPC, spatial learning, cavity volume

Traumatic brain injury (TBI) is a devastating disease with large social and economic impacts. However, despite these facts it has no standard pharmacological treatment. We hypothesized that the intravenous injection of multipotent adult progenitor cells (MAPC) in a rodent TBI model provides cellular protection leading to cognitive improvement. The rodents were tested for spatial learning using a Morris water maze at 28 days and 120 days after injury via a controlled cortical impact (CCI) to evaluate functional effects of the cell treatment (10 million/kg). In addition, we extracted the brains and measured volume loss by utilizing a computer tomography (CT) scan and SolidWorks software.

To calculate the cavity volume of injured rat brains we began by importing the CT scan data into SolidWorks in the point cloud format (.xyz). Scan tools were utilized to construct a mesh and then a surface. The surface included the cavity from injury, and its volume was calculated by filling the cavity with a solid that the software can accurately measure. To fill the cavity we first made a cap that covers the cavity along the brain surface using the 3D spline and filled surface features. Once the cap was made we create a solid using an extruded boss/base that goes through the entire cavity both below the original imported surface and above the cap surface we just created. We then cut this solid along both the imported and cap surfaces thereby removing everything except for what remains inside the cavity. The volume of the solid inside the cavity could then be measured. In the 120 day animals, results showed that the cavity volume is $0.12 \pm 0.07 \text{ cm}^3$ in rodents with the treatment compared to $0.03 \pm 0.01 \text{ cm}^3$ in those with CCI alone. In the 28 day animals, there is little change in volume loss as CCI alone averaged 0.04 cm^3 and treatment averaged 0.03 cm^3 .

The rodent behavior was tested using a Morris water maze and probe test. We measured the time and distance traveled by the rodents to determine spatial learning. The distance traveled was measured using ImageJ software. In the 120 day rodents, the data showed a significant difference ($p < 0.05$) between the CCI-alone and treatment group in both the time and distance to platform. The CCI-alone group showed an average time of 37 seconds and distance of 246 ± 28 inches, where as the treated group averaged 24 seconds and 171 ± 28 inches. There was no difference with treatment in the 28 day animals, as both averaged 35 seconds.

In conclusion, MAPC treatment is effective in improving cognitive behavior after TBI as measured by spatial learning at 120 days after treatment but not at 28 days. There are

significant differences in latency and distance traveled between the treated and untreated group. There are volume changes between treated and untreated but these changes are not significant. However, the larger volume loss in the treated group may be indicative of an underlying mechanism by which MAPC treatment improves spatial learning. Hopefully this work will lead to a better understanding of the mechanisms taking place with MAPC treatment, and one day MAPCs may be given as a therapy following TBI.

ABSTRACT

The influence of M73 metalloproteases on the *Bacillus anthracis* secretome

JENINE N. ZAIBAQ

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Class of 2015

Sponsored by: Michelle C. Swick, PhD, Theresa M. Koehler, PhD - Department of Microbiology and Molecular Genetics

Supported by: Theresa M. Koehler, PhD; University of Texas Health Science Center at Houston Medical School - Office of the Dean

Key Words: metalloproteases, *Bacillus anthracis*, secretome, virulence

Bacillus anthracis (*Ba*) secretes a large number of virulence factors during infection. Research has been focused on the three plasmid-encoded anthrax toxin proteins, but these proteins do not fully explain the pathology of anthrax disease. For example, the secreted protease Immune Inhibitor A1 (InhA1) of *Ba* has been associated with increased bacterial dissemination within the host and increased permeability of the blood brain barrier (D.V. Mukherjee, 2011). Two other secreted proteases, camelysin and TasA, are potential virulence factors. These M73 metalloproteases require zinc for activity and are produced by all members of the *B. cereus* group, including *Ba* (Grass, 2004). In strains of *B. cereus* associated with toxin-mediated diarrheal and emetic food poisoning, camelysin is able to cleave multiple host proteins (Dale & Koehler, 2012). Camelysin decreases InhA1 protein levels in *Ba* culture supernates, possibly through direct cleavage (K.J. Pflughoeft, 2011). In turn, InhA1 directly cleaves a large number of secreted *Ba* proteins, including the well-studied Lethal Factor (LF) component of anthrax toxin (Pflughoeft, Swick, & Koehler, in prep). Furthermore, TasA may affect protein levels of LF in the supernate (Pomerantsev, 2011). Because of their homology, TasA and camelysin may exhibit similar activities. We hypothesize that the M73 metalloproteases camelysin and TasA contribute to virulence by altering the levels of toxin proteins in the secretome. To determine the *in vivo* activity of TasA on LF, I compared the LF levels in cultures of the Sterne-like parent strain ANR-1, a *tasA*-null mutant, and a *tasA*-null complemented with *tasA* on an IPTG-inducible plasmid. Following growth in toxin-inducing conditions, I tested for the presence of LF in culture supernates using western blotting. I observed that LF protein levels in the supernate were increased in the *tasA*-null mutant when compared to the parent strain, but I did not observe complementation. Future studies will seek to restore the ANR-1 phenotype in the *tasA*-null mutant. To assess the *ex vivo* activity of camelysin, I successfully cloned an IPTG-inducible gene encoding recombinant camelysin with a GST tag at the amino-terminus. Compared to an uninduced control, solubilized *Ba* cells induced with IPTG expressed the GST-CalY fusion protein. Protein purification attempts are currently underway so that activity can be assessed, including direct cleavage of InhA1.



Undergraduate Students

ABSTRACT

Fracture Healing in Ovariectomized Sprague-Dawley Rats

SAMANTHA R. ALTEMUS

Texas A&M University

Class of 2013

Sponsored by: Catherine G. Ambrose, PhD, Department of Orthopedic Surgery

Supported by: Catherine G. Ambrose, PhD, Department of Orthopedic Surgery; University of Texas at Houston Medical School – Office of the Dean

Key Words: Fracture healing, ovariectomized, hypogonadism

There have been many studies linking the effects of hormone deficiencies, especially low estrogen, to osteoporosis. Our hypothesis was that osteoporosis delays fracture healing in ovariectomized (OVX) rats. The investigation of the relationship between hormone deficiency, osteoporosis, and fracture healing can provide new treatments and preventative measures for this disease.

Eight OVX and eight intact Sprague-Dawley rats were used in this study. Fractures were created in one femur of each rat, the bone was reamed, and a k-wire was placed inside to stabilize the fracture. Half the animals were left to heal for two weeks and the other half for four weeks. After sacrifice, the animals were imaged using quantitated CT to obtain bone mineral density (BMD) values. Both the intact and broken femurs were removed from the animals and fractured to obtain mechanical strength data. Mechanical testing was performed by using a 3-point bending technique along the midshaft and a compressive technique along the femoral neck of each bone.

The mechanical testing results showed that control females healed 27.3% over two weeks, while the OVX females healed 12.1%. The max load for the femoral necks in the OVX animals at two weeks was lower than the control animals, but this was not significant. The max load for the fractured limbs at two weeks was 14.5N in the OVX animals and 31.2N in the control animals. These values were both significantly lower than the max loads in the intact limbs and this trend continued in the four week group. Lumbar BMD averaged 572.23mg/cc in the control animals and 517.65mg/cc in the OVX animals at two weeks. The mean weight gain of the OVX females was 56g and 86.5g, while the weight gain in the control animals was 9.75g and 19g at two and four weeks, respectively.

The onset of osteoporosis was apparent at the two-week time point; the OVX females gained significantly more weight compared to the controls and the femoral neck tests showed that the max load was much lower in the OVX animals. This was also supported by the lumbar BMD data which showed lower values in the OVX animals at two weeks. Max loads in the fractured limbs showed that control females were stronger than the OVX females and overall, there was a delay in fracture healing in the OVX animals at two weeks. At this time, data collection is ongoing and more is needed to extend these findings to later time points.

ABSTRACT

Confirmation of Net1A Tyrosine Phosphorylation Sites

BRITTANY APPELBOOM

Wesleyan College

Class of 2013

Sponsored by: Jeffrey A. Frost, PhD, Department of Integrative Biology and Pharmacology

Supported by: Jeffrey A. Frost, PhD, Department of Integrative Biology and Pharmacology;
University of Texas at Houston Medical School – Office of the Dean

Key Words: Net1A, RhoA, Src, tyrosine phosphorylation

Rho GTPases are molecular switches that cycle between active, GTP-bound and inactive, GDP-bound states. Once active, they stimulate intracellular signaling to affect cytoskeletal organization, cell migration, and cell proliferation. Their activation is stimulated by Rho Guanine nucleotide Exchange Factors (RhoGEF), which catalyze GTP binding. Rho GTPase inactivation is promoted by Rho GTPase Activating Proteins, which stimulate GTP hydrolysis. Net1A is a RhoGEF that activates the RhoA subfamily of GTPases. Net1A was previously shown by our group to be phosphorylated by Src, which is a tyrosine kinase that is activated by many growth factors and plays a role in regulating RhoA activity. In that analysis five tyrosine phosphorylation sites were identified by mass spectrometry and phenylalanine substitutions were created for each site. The aim of this research project was to confirm the phosphorylation sites and to determine the effect of this phosphorylation on Net1A activity. Hela cells were transfected with FlagMyc-epitope tagged Net1A mutant plasmids plus active Src. The Net1A was then immunoprecipitated (IP) and tested for phosphorylation by Western blotting. From this work we were able to determine that the five sites previously identified were the only sites phosphorylated. The activity of Net1A was also tested using a GST-A¹⁷RhoA pull down assay. The FlagMyc epitope tagged Net1A and Net1A-5YF mutant were transfected +/- constitutively active Src. The results of this experiment showed that co-expression of active Src inhibited Net1A activity, and that the Net1A-5YF was not affected by Src. These data confirm that Net1A is phosphorylated by Src in Hela cells and indicate that this is a mechanism to downregulate Net1A activity. Future work will be aimed at identifying the specific Src phosphorylation sites that inhibit Net1A activity, and assessing the mechanism by which this blocks RhoA activation.

ABSTRACT

Color Adjustment Potential of Resin Composites in Simulated Class I Restorations: Colorimetric Study

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University of Texas at Austin

Class of 2014

Sponsored by: Magda S. Eldiwany, DDS, Joe C. Ontiveros, DDS, Rade D. Paravina, DDS, MS, PhD, Department of Restorative Dentistry

Supported by: Joe C. Ontiveros, DDS, Department of Restorative Dentistry

Key Words: Resin composite, Color adjustment potential (CAP)

Objective: To evaluate the color adjustment potential (CAP) of resin composites in simulated class I restorations using visual and instrumental method. **Methods:** The following resin composites (n=5) were used: - Test shades: A2 shades of TPH3 (TPH) and 3 competitors: Estelite \square Quick (ESQ), Filtek Supreme Ultra -Body (FSU), and Herculite Ultra (HCU); Control shade: A2 shade of Esthet-X (ESX). Disc-shaped specimens (D=10 mm, 2-mm thick) and “dual” specimens (outer ring (outer D=10 mm, with inner space of D=4 mm, 2-mm thick) were made. Each specimen was polished using PoGo disks for 40 seconds. Color evaluations were performed by means of non-contact spectroradiometer. A 1-mm circular area was measured. Instrumental color adjustment potential (CAP_I) was calculated as follows: $CAP_I = 1 - \frac{\Delta E_D}{\Delta E_S}$. Means and standard deviations were determined. The data were analyzed by analysis of variance. Fisher’s PLSD intervals for comparison of means were calculated at the 0.05 level of significance. **Results:** Single (s) and dual (D) specimens: color differences (ΔE^*) and instrumental color adjustment potential (CAP_I), are given in the table:

Comp.	ΔE_S	ΔE_D	CAP _I
TPH	3.5(0.4)	2.0(0.3)	0.41
ESQ	4.4(0.1)	2.9(0.1)	0.35
FSU	1.7(0.5)	0.7(0.1)	0.61
HCU	4.2(0.3)	3.9(0.2)	0.07

Specimen type (single-dual) and composite brand were found to be statistically significant for CAP_I ($p < 0.001$, power = 1.0). The same is true in the interaction of specimen type and composite brand for CAP_I. Fisher’s PLSD intervals for comparisons of ΔE^* values among specimen type and brands were 0.18 and 0.26, respectively. **Conclusion:** Color adjustment potential was specimen type (single-dual) and composite brand dependent. CAP_I decreased in the following order: FSU>TPH>ESQ>HCU.

ABSTRACT

Color Adjustment Potential of Resin Composites: Visual Assessment

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Supported by: Joe C. Ontiveros, DDS, Department of Restorative Dentistry

Key Words: Color adjustment potential (CAP), visual ratings

Objective: To use visual methods to evaluate the color adjustment potential (CAP) of resin composites in simulated class I restorations.

Methods: Four different composites from various manufacturers and one control composite were used. The shade of each composite that was compared and analyzed was the A2 shade. The following resin composites were used: TPH3 (TPH), Estelite Σ Quick (ESQ), Filtek Supreme Ultra –Body (FSU), and Herculite Ultra (HCU). The control shade was Esthet-X (ESX). A total of fifty specimens were made; twenty-five single composite specimens (D=10 mm, 2-mm thick) and twenty-five dual composite specimens (outer D=10 mm, with inner space of D=4 mm, 2-mm thick). Since the dual specimens contain an outer ring and an inner ring, the outer will be made of the control shade and the inner ring holes will be filled with one of the 4 test shades or the control shade. Each specimen was cured and polished for 40 seconds. Visual color evaluations were performed by five evaluators with normal color vision and superior color competency based on the test for color competency in dentistry. Specimens were placed on the floor of a viewing booth and observed in edge contact using 0/45° optical geometry, D65 illuminant and luminance of 1.000 lx. Color difference between test and control shades were graded on a scale from 1 (total mismatch) to 5 (perfect match) for all specimens. Visual color adjustment potential (CAP_V) was calculated as follows: $CAP_V = 1 - VR_D / VR_S$. After collecting data, the means and standard deviations were calculated. The data was analyzed by analysis of variance. Fisher’s PLSD intervals for comparison of means were calculated at the 0.05 level of significance.

Results: Single (s) and dual (D) specimens: visual ratings (VR) and visual color adjustment potential (CAP_V) are given in the table:

Composite	VR _S	VR _D	CAP _V
TPH	2.7 (0.3)	1.8 (0.4)	0.32
ESQ	3.7 (0.2)	3.0 (0.4)	0.19
FSU	1.9 (0.5)	1.3 (0.1)	0.33
HCU	3.4 (0.4)	2.8 (0.4)	0.15

Specimen type (single-dual), composite brand and their interaction were found to be

statistically significant ($P \leq 0.001$) for CAP_v . Fisher's PLSD intervals for comparisons of visual ratings were 0.23 and 0.33.

Conclusions: Color adjustment potential was dependent on both composite brand and specimen types (single or dual). CAP_v decreased in the following order: FSU > TPH > ESQ > HCU.

ABSTRACT

Activity of AtxA homologs in two *Bacillus cereus* group members

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Sponsored by: Theresa Koehler, PhD, Department of Microbiology and Molecular Genetics

Supported by: Molecular Basis of Infectious Disease NIH sponsored training grant T32
AI055449-07

Key Words: Activity levels, *atxA*, *lef*, *lacZ*, β -galactosidase assay

AtxA is the master virulence regulator in *Bacillus anthracis*, and is required for anthrax toxin synthesis and capsule expression. *B. anthracis* is a member of the *Bacillus cereus* group species that are characterized as Gram-positive, spore forming, rod-shaped, and facultatively anaerobic bacteria. While *B. anthracis* causes anthrax, some *Bacillus cereus sensu stricto* strains cause food-poisoning. Nevertheless, rare strains of *B. cereus* have been associated with more severe “inhalational anthrax-like” infections in humans and chimpanzees. *B. cereus* G9241 was isolated from a welder with a pulmonary anthrax-like illness. This unusual isolate possesses two virulence plasmids, pBCXO1 and pBC218, with homology to the *B. anthracis* virulence plasmid pXO1. Plasmid pXO1 encodes the anthrax toxin structural genes, as well as *atxA*. Each of the *B. cereus* G9241 plasmids contains an *atxA* allele. The alleles have 100% and 79% identity to *B. anthracis atxA*. The goal of this study is to compare the activity of the three *atxA* alleles: one from *B. anthracis* and two from *B. cereus* strain G9241. To alleviate potential species-specific gene expression differences, the alleles were cloned into plasmid vectors that allow *atxA* gene expression to be controlled by addition of IPTG to growing cultures. The constructs were introduced into a *B. anthracis* strain that has an AtxA-regulated promoter fused to *lacZ*, a gene encoding β -galactosidase. Results of β -galactosidase assays indicate that the activity of the proteins encoded by the *B. cereus* G9241 *atxA* genes is significantly less than that of the *B. anthracis* AtxA. Future directions of this project will be to determine whether the *B. cereus* G9241 *atxA* alleles are being expressed using RT-PCR, and whether the resulting protein is stable using immunoblotting.

ABSTRACT

Incorporation of hyaluronic acid into the *Staphylococcus aureus* matrix

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Supported by: Molecular Basis of Infectious Disease NIH sponsored training grant T32
AI055449-07

Key Words: Hyaluronic Acid, fluorescent HA, *Staphylococcus aureus*, biofilm matrix

Staphylococcus aureus is one of the leading causes of complications among patients who have received joint or catheter implants resulting in serious infections, such as osteomyelitis and endocarditis. These infections result from the growth of *S. aureus* on surfaces, which is termed a biofilm. A biofilm is surrounded by a protective matrix composed of self-generated extracellular material. This study focused on the incorporation of hyaluronic acid (HA) into the *S. aureus* matrix. HA, a non-sulfated glycosaminoglycan, plays a role in inflammation control in humans and as a virulence factor in *Streptococcus pyogenes*. However, its involvement in *S. aureus* pathogenicity has not been well characterized. We hypothesize that HA is incorporated into the *S. aureus* matrix. The Stains-All colorimetric assay was used to measure HA association with the *S. aureus* matrix. The greatest association of HA with the *S. aureus* matrix was observed when overnight liquid cultures were grown in the presence of HA and the cells were washed three times before treatment. To examine the association of HA with the *S. aureus* biofilm matrix, biofilms were grown in the presence of fluorescent HA and observed with a fluorescence microscope. Specifically, polymethylmethacrylate (PMMA) discs were grown in 60% glucose-free tryptic soy broth containing 0.2% glucose, and fluorescent HA (1 mg/ml) in a 24-well plate for 48 hrs at 37°C. Microscopic analysis suggests that HA was incorporated into the biofilm matrix. We will continue to examine the role HA plays in *S. aureus* biofilm formation. *S. aureus* cells can synthesize their own hyaluronidase, which appears to be a virulence factor and may be necessary for incorporation of HA into the matrix.

ABSTRACT

Comparison of Phagocytosis in Wild Type and C3aR $-/-$ Splenocytes

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Supported by: Molecular Basis of Infectious Disease NIH sponsored training grant T32 AI055449-07

Key Words: *L. monocytogenes*, C3a, complement, phagocytosis

Listeria monocytogenes, a food-borne pathogen, infects the host through induced phagocytosis of epithelial cells to enter the blood stream and enter the liver and spleen. The cell-mediated response is activated and thus makes this organism an optimal model for the study of the immune response, in particular, complement C3a and its corresponding receptor, C3aR. A previous study from this lab had shown that more *L. monocytogenes* was recovered from C3aR $-/-$ spleens than from WT spleens. It was hypothesized that the C3aR $-/-$ splenocytes have a decreased phagocytic ability than the WT splenocytes. To test this hypothesis, spleens were removed from naïve wild type and C3aR $-/-$ mice, homogenized, and single cell suspensions were plated. These cells were infected with *L. monocytogenes* for 3, 24 and 48 hours. There were no significant differences in recovered CFU/mL in either the WT or C3aR knockouts at any of the time points tested. Since TNF- α is released upon infection to promote macrophage activation, an ELISA was performed looking for both TNF- α and IL-10, which inhibits macrophage activation, in all time points and samples to clarify macrophages were being signaled to activate. There were no differences in TNF- α levels between the WT and C3aR $-/-$ samples, and no IL-10 was detected. There appears to be no difference between the WT and C3aR $-/-$ splenocyte ability to phagocytose *L. monocytogenes* and further studies will be needed to determine why C3aR $-/-$ mice have a higher *L. monocytogenes* burden in their spleens.

ABSTRACT

A17 Amacrine Cells in Primate Retina Contain CART

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Supported by: David W. Marshak, PhD, Department of Neurobiology and Anatomy**;
University of Texas at Houston Medical School – Office of the Dean**

Key Words: CART, primate, A17, retina

The neuropeptide Cocaine- Amphetamine- Regulatory Transcript (CART) has been localized to the brain and the retina. While there has been considerable research on its role in the brain, the functions of CART in the retina are not known. CART has been localized to ganglion cells of rat retina, and the goal of these experiments was to localize it in the primate retina. We used two antisera: goat-anti-human-CART-28-116 and rabbit-anti-rat/mouse/bovine-CART-55-102. Immunolabeling was done on tissue fixed in 0.05% glutaraldehyde and 4% paraformaldehyde or else on tissue fixed in 4% paraformaldehyde. The dilutions used were: 1:200, 1:500, 1:1000, and 1:2000. The 1:500 dilution of the goat-anti-CART antibody and the 1:2000 dilution of the rabbit-anti-CART antibody labeled the best. Both antisera labeled the same amacrine cells; the results with the two fixatives were similar. The morphology of the labeled neurons was analyzed by confocal microscopy in vertical, 50 μm vibratome sections. We observed that the CART-labeled cell bodies in the inner nuclear layer (INL) were typically next to each other, a finding suggesting that two types of amacrine cells had been labeled. However, the two types could not be distinguished; both had thin, varicose dendrites in the inner plexiform layer (IPL). Statistical analysis was performed using a 2-normal-mixture model to describe the location of the varicosities in the IPL. Depth was defined as a percentage of the distance from the INL to the ganglion cell layer. From 0-60% through the IPL, there was a relatively low density of CART-labeled varicosities. There was a major peak at 82.5% with a standard deviation of 10.1 indicating that CART varicosities are most prevalent in S5, the innermost stratum of the IPL. Most labeled dendrites gradually descended to S5 with a curving trajectory. Others ran for approximately 35 μm , on average, in the outermost stratum of the IPL before descending to S5; a few dendrites descended there directly. These findings suggest that CART labels two types of ON-center amacrine cells that have similar functions. Based on their morphology, the CART-labeled cells appear to be the A17, or spidery type, amacrine cells, which are known to receive input from rod bipolar cells and to make reciprocal, inhibitory synapses with them. Using antibody to CART, it will be possible to label the entire population of these cells and to learn more about their functions.

*each author contributed equally to this work

ABSTRACT

Antibody response to LCL protein in Multiple Sclerosis

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Supported by: John W. Lindsey, MD; Department of Neurology; University of Texas at Houston Medical School - Office of the Dean

Key Words: Multiple sclerosis, Epstein-Barr, Lymphoblastoid cell line

Epstein-Barr virus (EBV) infection has been shown to be associated with multiple sclerosis (MS). The antibody response to EBV antigens, particularly Epstein-Barr nuclear antigen-1 (EBNA-1,) is increased in those with MS. The antibody response to a range of EBV antigens has been studied, but not to antigens present in lymphoblastoid cell lines (LCLs) specifically. To study the antibody response to LCL protein in MS, a Western blot containing LCL protein was incubated with sera from 41 pairs of MS patients and matched controls. For most subjects, there was a single prominent band at 75kD with occasional other minor bands. We quantified the antibody response to the 75 kD band by densitometry compared to a reference IgG. To test whether EBNA-1 was present in this protein, a Western blot containing LCL and B95.8 cell proteins were incubated with anti-EBNA-1 antibodies. The antibody response to the 75kD LCL protein was higher in MS (median 16.0 mg/ml) than in controls (median 9.2 mg/ml) ($p=0.02$, rank sum test). Western blots with a monoclonal EBNA-1 antibody had a band at the same molecular weight as serum. This further supports the theory of the increased immune response to EBV in MS and suggests that EBNA-1 is the main antigen involved.

ABSTRACT

Deciphering the Role of Metal Ions in Learning and Memory

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Sponsored by: M. Neal Waxham, PhD, Department of Neurobiology & Anatomy

Supported by: M. Neal Waxham, PhD, Department of Neurobiology & Anatomy; University of Texas at Houston Medical School – Office of the Dean

Key Words: Postsynaptic Density, Scaffold proteins, EDTA, Immunogold Labeling

Neurons communicate with each other through specialized junctions called synapses, which are between the presynaptic axons and postsynaptic dendrites. The postsynaptic density (PSD) is a disc-shaped protein complex located on the postsynaptic membrane, which specializes in regulating synaptic transmission. PSD structure is plastic, changing through development and neural activity, which is hypothesized to form the basis of learning and memory. Zinc and other metal ions are important for proper function of many proteins in the brain, including those found as integral PSD proteins. The binding of Zinc to PSD scaffold proteins is known to influence their interactions with other PSD-associated proteins. Understanding the role of metal ions in modifying the structure of PSDs can provide profound insight towards learning and memory and potentially in disorders such as autism and Alzheimer's disease. We investigated the morphologic changes in PSDs isolated from rat forebrain when depleted of metal ions using the chelator ethylenediaminetetraacetic acid (EDTA). After PSDs were treated with EDTA, immunogold labeling for PSD proteins (PSD-95, Shank2, Shank3, SAP102, CaMKII, and Actin) was used to analyze the impact of metal ion removal on protein amount and distribution. Depleting PSDs of metal ions resulted in an overall thinning of the PSD structure and a quantitative reduction in (need to state which proteins). Additionally, when the protein distribution was analyzed, we found a statistically significant increase in the clustering of certain proteins in the EDTA-treated PSDs. Removal of metals causes PSD thinning presumably by removing a subset of proteins and in so doing reveals additional proteins in the core structure that can be gold labeled. Our data confirms the importance of metal ions in maintaining PSD structure; suggesting metal ions are essential for proper synapse function.

ABSTRACT

Assessing the cytopathogenicity of enteroaggregative and diffusely adhering *E. coli* by flow cytometry

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Sponsored by: Pablo Okhuysen, MD, Department of Internal Medicine

Supported by: Pablo Okhuysen, MD, Department of Internal Medicine

Key Words: Enteroaggregative, diffusely adhering, *E. coli*, intestinal epithelial cells

Background: Enteroaggregative (EAEC) and diffusely adhering *E. coli* (DAEC) cause diarrhea in children living in developing countries. Traditionally, the diagnosis is made by examining the adherence patterns onto intestinal epithelial cells (IEC) by light microscopy which is time consuming and reader dependent. We sought to determine if the use of flow cytometry could measure and quantitate the adherence and cytopathogenicity of EAEC and DAEC isolates from adults and children with diarrhea.

Methodology: Individual EAEC and DAEC isolates were labeled with the fluorescent dye PKH67. Labeled bacteria were then added to human HEP-2 epithelial cells labeled with the fluorescent dyes PKH26 (to measure viability) and a dye specific for Annexin V (to measure apoptosis). We examined the proportion of cells that adhered to IEC, as well as the proportion of IECs that were alive, dead or undergoing apoptosis.

Results: 61 isolates were tested (36 EAEC and 25 DAEC) for adherence and cytopathogenicity and compared to control strains (EAEC and DAEC and the non-pathogenic HS). The non-pathogenic control strain did not induce apoptosis or cell death, while the reference EAEC 042 and DAEC adhered to IECs. EAEC 042 induced cell death while the reference DAEC was associated with apoptosis. Thirty five of the 61 isolates (57%) caused cell death. The proportion of isolates causing cell death was similar among the EAEC and DAEC groups and was independent of the presence of known virulence genes.

Conclusions: Flow cytometry provides a method to quantitate the adhesion of EAEC and DAEC to IECs. The observation that cytopathogenicity was independent of the presence of known virulence genes suggests that additional factors are associated with EAEC and DAEC pathogenicity.

ABSTRACT

Aortic Remodeling Following Transverse Aortic Banding in Mice is Attenuated with AT1 Receptor Blockade

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Supported by: Dianna Milewicz, MD, PhD, Director, Medical Genetics, Department of Internal Medicine; University of Texas at Houston Medical School – Office of the Dean

Key Words: Aortic aneurysm, transverse aortic banding, TGF- β signaling

Background: A novel mouse thoracic aortic aneurysms and aortic dissections (TAAD) model was recently developed in laboratory by using transverse aortic banding (TAB) to create biomechanical stress. The goal of this project is to characterize and assess the molecular mechanisms underlying ascending aortic remodeling in response to increased biomechanical pressure.

Methods and Results: Twelve-week-old male mice underwent TAB for 2 weeks. Mouse aorta dilatation was observed and angiotensin signaling was activated. Inflammatory response was significantly increased in the ascending aortas of TAB mice compared to sham mice with increased levels of inflammation markers IL-6 and MCP-1 as well as macrophage infiltration. TGF- β signaling was activated in TAB mice compared to sham mice with increased mRNA levels of TGF- β 1 and TGF- β 2 and phosphorylation of Smad2/3 proteins. Losartan, the AT1 receptor blocker, significantly reduced inflammatory response and inhibited TGF- β activation in TAB mice.

Conclusion: Increased biomechanical forces on the ascending aorta induced structural remodeling and aortic dilatation in association with an aortic inflammatory response and the activation of the AT1 receptor.

ABSTRACT

Use of Thromboelastography to Predict Clot Composition and Subtype in Acute Ischemic Stroke

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Sponsored by: James Grotta, M.D., Professor and Chair Department of Neurology

Supported by: James Grotta, M.D., Professor and Chair Department of Neurology and The University of Texas Health Science Center at Houston Medical School - Office of the Dean

Key Words:

Introduction Tissue Plasminogen Activator (tPA) is the only FDA approved treatment for acute ischemic stroke (AIS). Following t-PA therapy only about 20% of patients experience rapid clinical improvement, and thus there exists a potential for improvement of current treatment guidelines. There is evidence to suggest that the efficacy of tPA-induced thrombolysis is dependent on the relative proportions of constituents that make up the target thrombus, or simply, clot subtype. Thromboelastography (TEG) provides an integrated assessment of coagulation in real time and can be used to discriminate between platelet and enzymatic contributions to clot formation. However, the relationship between TEG values and clot subtype in AIS has yet to be explored. Using TEG to predict clot subtype and tPA responsiveness could lead to more efficient, customized treatment for AIS patients.

Methods AIS patients who received tPA within 4.5 hours of symptom onset were enrolled. Blood was collected for TEG analysis prior to tPA treatment and 10 minutes after tPA bolus. In addition, a clot specimen was obtained from each blood sample and histological analysis was performed. The specimens were then categorized as heterogeneous or homogeneous, and erythrocyte predominant (red) or fibrin/platelet predominant (white) based on their microscopic histological appearance. TEG values were compared to clot composition as a categorical variable, either erythrocyte predominant or platelet-fibrin predominant. The comparison was also made between TEG values and estimated component-percentage as a continuous variable, e.g. %RBC area, %platelet-fibrin area.

Results 10 patients were enrolled, all of who had complete pre-tPA TEG results. There was no correlation found between TEG values and white or red clots, nor was there any connection between TEG values and homogenous or heterogeneous clot type.

Conclusion TEG values in AIS patients may help determine clot subtype but based on this small sample cannot independently define the type of clot or predict clinical response to tPA. A further study of a larger patient sample is necessary.

ABSTRACT

Noncanonical Function of Seryl tRNA Synthetase in Zebrafish Angiogenesis

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Sponsored by: Eric C. Swindell, PhD, Department of Pediatrics

Supported by: Eric C. Swindell, PhD, Department of Pediatrics ; University of Texas at Houston Medical School - Office of the Dean

Key Words: Serine, tRNA Synthetase, Angiogenesis

Aminoacyl tRNA synthetases have been known for their enzymatic function of binding amino acids to their correspondent tRNA. Recently, however, many tRNA synthetases have shown additional functions, ranging from apoptosis regulation to retroviral particle assembly. Among these synthetases, the Seryl tRNA synthetase (SARS) has been shown to have a non-canonical effect on angiogenesis in addition to its aminoacylation activity. Using the hi3817 zebrafish mutant, in which no SARS mRNA is produced, the effect of the absence of SARS was observed. The mutant zebrafish embryos display aberrant blood vessel formation in the intersegmental vessels (ISV); however, by injecting wild type SARS mRNA with defective aminoacylation activity, the mutant phenotype can be rescued. The hi3817 mutant is crossed with GFP transgenic fish so that blood vessel can be visualized through fluorescent microscopy. Rescue was defined as having phenotypically normal blood vessels while being genotypically mutant as determined by PCR. Several mRNA injection constructs were created to eliminate specific regions of the SARS protein. By determining which of these constructs rescue the mutant phenotype, we can identify which region of the SARS protein is responsible for its role in angiogenesis. In addition, by studying other types of rescue constructs, the portion of SARS responsible for the angiogenic activity can be further localized and future studies on the mechanism of the angiogenic activity of SARS can be pursued.

ABSTRACT

Diagnosis and Treatment of Pediatric Bipolar Disorder

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Supported by: Oscar Bukstein, MD, Director of Child and Adolescent Services, Department of Psychiatry and Behavioral Sciences; University of Texas at Houston Medical School – Office of the Dean

Key Words: pediatric bipolar disorder, diagnosis, treatment

Bipolar disorder is a psychiatric condition that is characterized by episodes of mania or hypomania paired with episodes of depression. The diagnosis, prevalence, and treatment of this illness in children and adolescents are controversial and disputed. Thus, it was determined that a more comprehensive assessment and treatment plan should be undertaken to provide proper diagnosis and rehabilitation through a Bipolar Program for Adolescents and Children (B-PAC). The Kiddie Schedule for Affective Disorders and Schizophrenia (KSADS) was chosen to be incorporated into an inter-rater system in order to properly diagnose pediatric bipolar disorder. Also, several measures such as the Young Mania Rating Scale (YMRS) and Quick Inventory of Depressive Symptomatology (QIDS) were chosen to provide an ongoing assessment of the patient's symptoms. If diagnosed, a patient will be subjected to a treatment algorithm that includes a combination of both pharmacotherapy and psychotherapy. Pharmacotherapy includes exposing the patient to atypical antipsychotic drugs and/or mood stabilizing drugs. Psychotherapy includes the practice(s) of cognitive-behavioral therapy, dialectical behavioral therapy, and/or family-focused therapy. Pending the analysis of specific outcomes, B-PAC has the potential to help numerous children and adolescents struggling with bipolar disorder that decide to seek medical care.

ABSTRACT

Sse1 and its role in polyglutamine protein aggregation in *S. cerevisiae*

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Supported by: Kevin Morano, PhD, Department of Microbiology and Molecular Genetics;
University of Texas at Houston Medical School - Office of the Dean

Key Words: Protein aggregation, chaperones, nucleotide exchange factors, HSP70, Sse1

Stresses caused by heat, pH and gene expression can lead to protein misfolding, protein aggregation and cell death. Aggregation of mutant proteins in nerve cells can cause human diseases such as Alzheimer's disease and Huntington's disease. Huntington's disease is caused by a mutant protein that has a variable length polyglutamine (polyQ) residue that causes misfolding and aggregation, which increase as the length of the residue increases.

Every organism has methods for handling protein misfolding. In the budding yeast, *Saccharomyces cerevisiae*, a family of chaperones helps to fold proteins into their native forms. These chaperones of the family Heat Shock Protein (Hsp) 70 work with the help of co-chaperones such as Hsp40s and many nucleotide exchange factors (NEFs). Hsp40s bring the unfolded protein to the Hsp70s while the NEFs attach to the ATPase binding domain, causing a conformational change that serves to release the native protein and the used energy source (ADP).

Protein folding and aggregation of polyQ residues have been shown to be dependent on cytosolic Hsp70s and can be easily modeled in *S. cerevisiae*. The NEF contribution to Hsp70-dependent folding of polyQ residues has not been widely studied. Of the four yeast cytosolic NEFs, Sse1, Sse2, Fes1 and Snl1, Sse1 is considered the most dominant and was the focus of my study. Additionally, Sse1 has a close human homologue, Hsp110, allowing its study in yeast to be applicable to understanding Huntington's disease in humans.

To determine the role of Sse1 in polyQ folding, I integrated four different glutamine-containing stretches – 25, 46, 72 and 103 – into the genome of two parental strains, one lacking Sse1 and one expressing it. I modulate the expression using an inducible GAL1 promoter. Additionally, I transformed p416-TEF-SSE1 into a strain already expressing SSE1 thereby overexpressing the gene.

In a serial dilution growth assay, both the wild type strain and $\Delta sse1$ strains exhibited increased toxicity as the polyQ residues increased in length, suggesting that there was no significant difference between WT and $\Delta sse1$.

Overexpressed Sse1 cells showed a similar phenotype to those not expressing SSE1, implying that excess of Sse1 in the cell did not alleviate polyQ-dependent toxicity.

ABSTRACT

Photoperiod-Regulated Plasticity of Retinal Physiology

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Supported by: NIH-NEI EY018640 to C.P.R. and EY010608 to the Dept. of Ophthalmology;
University of Texas at Houston Medical School – Office of the Dean

Key Words: Dopamine, Melanopsin, Photoperiod, Circadian Clock, Retina

A large body of evidence indicates that retinal physiology and function are regulated on a daily basis by light-dark adaptive mechanisms as well as endogenous processes. For instance, the release of dopamine, a catecholamine (CA) that regulates light adaptive processes, by dopaminergic amacrine cells is controlled by photoreceptor response to light and by the circadian clock inherent in the retina. Both rod and cone photoreceptors and recently discovered intrinsically photosensitive retinal ganglion cells (ipRGCs), which express the photopigment melanopsin, participate in the control of dopamine release by light. By these processes, dopamine secretion is elevated during the day and diminished at night. Recent evidence indicates that dopamine secretion is controlled by the photoperiod, the duration of the daily light phase. The daily rhythm of dopamine release is of higher amplitude under long photoperiods (18 h light/day) compared to that of short photoperiods (6 h light/day). In this research, we investigated whether a photoperiodic change in dopamine release reflected a change in the expression of tyrosine hydroxylase (TOH), the rate-limiting enzyme in the synthesis of dopamine, and/or a change in the expression of melanopsin in ipRGCs. CBA/Ca mice adapted to long or short photoperiods (> 1 month) were anesthetized and euthanized by cervical dislocation. Retinas were dissected and fixed. Retinas, cryo-sectioned or kept whole, were immunostained for TOH and melanopsin. The density and immune-sensitivity of dopaminergic amacrine cells and ipRGCs were quantified using fluorescence and confocal microscopes. The density of ipRGCs was significantly higher by 30% in the long photoperiod ($p = 0.017$; $n = 7$). Both the density of dopaminergic cells and the expression of TOH were 30% greater under the long photoperiod although the increases were not statistically significant. The average melanopsin expression in ipRGCs was similar under both photoperiods. Finally, dopaminergic amacrine cells were found to contain more varicosities extending deeper into the inner plexiform layer in longer photoperiod. This data clearly demonstrates that the physiology of both ipRGCs and dopaminergic amacrine cells is affected by the photoperiod. The overall trend is higher expression of TOH, melanopsin, and their respective cell density under the longer photoperiod. Future experiments will confirm this data and determine whether this plasticity is caused by the formation of new cells or by the up regulation of melanopsin and TOH expression in cell subtypes that typically show low immunoreactivity for melanopsin (M3, M4, and M5 subtypes) or TOH (CA2 subtype). Furthermore, future experiments in melanopsin knockout mice will also help determine whether melanopsin is required for the photoperiod-regulated plasticity of ipRGCs and dopaminergic cells.

ABSTRACT

Behavioral Effects of Methylphenidate in Children with ASD and symptoms of ADHD

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Key Words: ASD, ADHD, psychostimulant

Children with Autism Spectrum Disorder (ASD) can also show symptoms of Attention Deficit Hyperactivity Disorder (ADHD). With numerous children having this comorbidity, little is known about the treatment of both disorders and its effects on behavior. This study examined the effectiveness of a range of doses of an extended release psychostimulant medication, methylphenidate (MPH), relative to behavioral effects in children with ASD and ADHD. Thus, the goals of this study were to observe if treatment resulted in improved parent and teacher behavioral ratings and if higher doses of MPH were associated with consistent improvements in behavioral functioning. The sample consisted of 24 children, who met DSM-IV-TR criteria for ASD on the Autism Diagnostic Interview, Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) while also displaying significant symptoms of ADHD. The effects of MPH on parent and teacher ratings such as Connors, ACTeRS, and ABC (parent only), were investigated using a within-subject, crossover, placebo-controlled design. Parent and teacher ratings reported significant decline in symptoms of ADHD and oppositional behavior as well as improvements in social skills. Trend analysis revealed that MPH dose response was linear (e.g., as MPH doses increase symptoms of ADHD decrease). Findings suggest that extended release MPH is associated with significant behavioral improvement in home and school settings in children with ASD and symptoms of ADHD.

ABSTRACT

Systemic sclerosis: A complex genetic disease

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Key Words: Systemic sclerosis, genome-wide association study, candidate gene study, human leukocyte antigen (HLA), STAT4, IRF5, CD247

Systemic sclerosis (SSc) is a fibrotic and autoimmune disease characterized clinically by skin and internal organ fibrosis and vascular damage and serologically by the presence of circulating autoantibodies. Although etiopathogenesis for this disorder is not yet well understood, genetic contributions appear important after considering family and twin studies. Two strategies, the candidate gene study and the genome-wide association study (GWAS), have been used to reveal a number of genes associated with SSc and/or its clinical and serological subsets. In this paper, the major genes of SSc are reviewed, including HLA genes, STAT4, IRF5, CD247, and more. The most recent GWAS are taken into account along with robust candidate gene studies. An attempt to categorize the genes according to function is made with the aim of shedding light on the pathways contributing to SSc pathogenesis.

ABSTRACT

Relapse vs. Reinfection of Patients with *Clostridium difficile* Infection

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Supported by: Molecular Basis of Infectious Disease NIH sponsored training grant T32
AI055449-07

Key Words: *Clostridium difficile*, recurrent *C. difficile* infection, ribotyping

Clostridium difficile infection (CDI) is a common hospital-acquired diarrheal disease in the United States. While treatment with vancomycin or metronidazole cures the majority of cases, there is still a high recurrence rate of 15-25%. Initial studies showed that approximately half of the patients with recurrent CDI were infected with two different strains when a patient's first and second infections were compared suggesting re-infection. It is not known if in fact multiple strains were present initially and these cases actually represent relapses. In this study we employed PCR ribotyping to type *C. difficile* strains in patients with recurrent CDI to determine if the strains causing second bouts were present or absent from the initial infection. Using PCR ribotyping we compared up to ten isolates from each infection (initial bout and recurrent bout) in order to distinguish between reinfection and relapse.

Our study included eighteen patients from St. Luke's Episcopal Hospital in Houston, TX with recurrent CDI from 2007-2012. *C. difficile* was cultured on Cycloserine Cefoxitin Fructose Agar (CCFA) from patient stool samples after alcohol shock. As many as ten separate colonies were selected from each patient sample and confirmed as *C. difficile* by PCR. PCR ribotyping was performed for each colony. Cluster analysis was performed by using SAS (v10, Cary, NC) to identify the degree of association between the strains is maximal if they belong to the same group and minimal otherwise. Proportions were calculated for categorical variables using the chi square test. The colonies were compared using a dendrogram from this data. 94.4% of the patients had more than one ribotype present in their samples. Based on molecular typing, we categorized 33% of cases as relapse, 39% as reinfection, and 28% as undetermined until further analysis. Our results show no significant difference between relapse and reinfection rates by strain typing. However, the rates determined by strain typing were different than the rates calculated by defining relapse as an infection occurring within eight weeks of the primary infection, showing that it is important to analyze infections with molecular methods when evaluating recurrent CDI cases. In addition, the high rate of mixed infections indicates that future strain typing studies should test several colonies.

ABSTRACT

Alternative Splicing Regulates Ankyrin Protein Diversity

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Key Words: Ankyrin, alternative splicing, β -spectrin.

Background: Ankyrins make-up a family of adaptor proteins that mediate the binding and localization of membrane proteins to the spectrin/actin-based membrane skeleton. Three genes: *ANK1*, *ANK2*, *ANK3* encode three different polypeptide ankyrin-R, ankyrin-B, and ankyrin-G, respectively. A typical ankyrin protein consists of three functional domains: the membrane-binding domain interacts with ion channels and transporters, spectrin-binding domain binds to β -spectrin, and the death domain and C-terminal domain function as a regulatory domain. A plethora of functionally diverse ankyrin proteins are generated by alternative splicing of three ankyrin genes. Alternative ankyrin isoforms display altered binding properties and differential subcellular localization. In addition, alternative splicing tailors ankyrin polypeptides to the different functional demands in different tissues. The purpose of this project is to identify and characterize novel ankyrin-B and ankyrin-G transcripts expressed in different mouse tissues.

Methodology: Reverse-transcriptase PCR was performed on mRNA isolated from mouse brain, cerebellum, heart, kidney and lung. Five primer sets spanning the membrane-binding domain of ankyrin-B and ankyrin-G were designed to subdivide the transcripts into overlapping sections of 600 to 800 bp in length. Novel transcripts were resolved on a 2% agarose gel, ligated into pCR2.1 TOPO vector, and sequenced. Quantitative real-time (qt)-PCR analysis was employed using exon-exon boundary spanning primers to measure the expression of specific alternative transcripts in the five tissues.

Conclusion: Three novel alternative ankyrin-G splice variants were identified. Two variants were missing exon 28 that encodes the minimal binding domain for β -spectrin. The third transcript lacked exon 23; the functional significance of this coding region has yet to be identified. Qt-PCR analysis demonstrated that transcripts lacking exon 28 are more abundant in brain compared to the other tissues. In contrast, expression of the transcript lacking exon 23 is most abundant in lung. These results demonstrate that each tissue expresses a significant diversity of alternative ankyrin mRNA transcripts. To address the functional significance of these novel transcripts, future investigations will include assessing the binding capacity of each transcript to the cytoskeletal protein β -spectrin.

ABSTRACT

A Prospective iPad-Based Screening Tool for mTBI

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0924636; University of Texas at Houston Medical School – Office of the Dean

Key Words: saccades, mild traumatic brain injury, postconcussion syndrome

In the United States, over 1.5 million people experience a traumatic brain injury each year, and up to 75% of those injuries are considered mild traumatic brain injury (mTBI). In approximately 20-30% of patients, mTBI causes persistent cognitive symptoms, but these cognitive sequelae are difficult to predict or detect by clinical measures of trauma severity such as GCS (Glasgow Coma Score), loss of consciousness, or degree of post-traumatic amnesia, or by traditional neuroimaging techniques such as CT scans, MRI scans, and EEG.

Previous eye-tracking studies suggest that mTBI measurably affects voluntary oculomotor function, and that differences in oculomotor function between mTBI patients can predict which patients will suffer from postconcussion syndrome (PCS). However, traditional eye-tracking devices are bulky and not easily portable; thus, we propose the use of a portable touch-based analogue of saccade and antisaccade tasks on a tablet computer. We hope to demonstrate that these reflexive pro-point and voluntary anti-point tasks are sufficiently sensitive for discriminating between mTBI patients and non-mTBI patients.

Mild TBI patients ($n = 3$) and orthopedic control patients ($n = 1$) were recruited from the ER of Memorial Hermann Hospital within 24 hours of injury. Normal controls ($n = 3$) were also recruited. Participants under the age of 18 or under the influence of alcohol were excluded from the study. Immediately following recruitment, we assessed subjects on pro-point and anti-point touch tasks. Preliminary results suggest that mTBI patients do not differ from orthopedic patients on pro-point (1 ms) but perform more poorly on anti-point touch tasks (207 ms). Orthopedic patients were slightly slower than normal control groups on both pro-point and anti-point tasks (153 and 133 ms, respectively). Future longitudinal studies would be important to test whether differences detect or predict PCS. This study is currently in progress.

ABSTRACT

Photoreceptor Coupling Mediated by Connexin35 in the Goldfish Retina

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Key Words: sensory system, retina, photoreceptor coupling, gap junctions, connexin, circadian rhythms

Photoreceptors in the retina are responsible for detecting photons and transducing light energy into neural signals that eventually are transmitted to higher brain areas. There are two types of photoreceptors named rods and cones that operate under different lighting conditions. Rods are responsible for dim light (scotopic) vision, while cones are responsible for bright light (photopic) vision. However, electrophysiological evidence indicates that rods and cones can mix signals with each other or photoreceptor cells of the same type through electrical synapses or gap junctions, a phenomenon called electrical coupling. It is believed that most of photoreceptor electrical coupling occurs in the outer plexiform layer (OPL), where photoreceptors terminals contact second order neurons. Recent anatomical evidence indicates that connexin35 (Cx35), or its mammalian homolog Cx36, gap junction forming protein is expressed in the OPL, more specifically in the close vicinity of the cone pedicles and their long evaginating processes named telodendria. The aim of this project was to label single cone pedicles in order to determine whether Cx35 is associated with cones and/or rods. Cones were labeled with the fluorescent dye Lucifer yellow (LY), which was either iontophoresed into Goldfish (*Carassius auratus*) cones during whole-cell patch clamp recording of their light responses, or bath-applied onto isolated retinas - a technique that allows LY to diffuse into injured cells, including cones. Triple-immunohistochemical staining was performed on formaldehyde-fixed retinas (sectioned or whole-mounted) with antibodies against LY (cone body/ telodendria), Cx35 and cone arrestin (entire cone population). The stained retinal tissues were observed using confocal microscopy. We found intense Cx35 labeling in the OPL clearly associated with cone pedicles and telodendria. Specifically, Cx35 plaques were found at areas of contact between LY+ and LY- cones along the telodendria, providing evidence for cone-cone coupling. In addition, many Cx35 plaques were found along the telodendria and on the surface of cone pedicles, indicating their involvement in rod-cone coupling. Finally, small Cx35 plaques were clearly unassociated with either cone pedicles or telodendria, suggesting they were located in rods. Our results indicate that Cx35 is the likely anatomical substrate for cone-cone, rod-cone and rod-rod electrical coupling in the Goldfish retina.

ABSTRACT

Differentiation of Chondrocytes From Bone Marrow Stem Cells

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Supported by: Pauline Jackie Duke, PhD, Department of Orthodontics

Key Words: Bone marrow stem cells, hyaline cartilage cells, anti-type II collagen antibody, chondrocytes

Background Bone-forming cartilage cells derived from mouse bone marrow stem cell provide sufficient amount of bone to repair a significant portion of bone defects. Such an approach to tissue engineering to form cartilage cells is a promising field for the clinical use.

Objective The objective was to differentiate mouse bone marrow stem cells (mBMSC) into hyaline cartilage cells on Silastic membranes forming the matrix produced by chondrocytes with an immunohistochemistry test for collagen type II.

Method Femurs and tibias from two C57BL adult female mice were dissected away from surrounding tissue and rinsed with PBS. Both ends of the bones were removed, and a small needle (27G) was used to inject medium into the bones to flush out bone marrow cells. Collected cells were centrifuged, the supernatant was discarded, and the cells were suspended in a non-differentiating medium. The cell suspension was placed in two T25 flasks and incubated under standard conditions (37°C, humidity, 5% CO₂). Half of the medium was replaced every other day. Loose cells were removed with the medium withdrawal. After the cells neared confluency, they were rinsed with PBS, and trypsinized with 0.05% Trypsin-EDTA. The cells were placed in Silastic membrane domes with differentiating medium, BGJb-Fitton Jackson Modification with 10% FBS, 1% Pen-Strep, and 150 µg/ml ascorbic acid. There was the absence of CO₂ for first two hours of incubation. The cells were stained with Alcian Blue at pH11.

Results Cell aggregates formed in the non-differentiating medium, and they actively divided for two weeks. The current method used to flush out the bone marrow stem cells yielded more retention of cells compared to the previous method that used collagenase and disperse to dissolve the bones. Cells on membranes aggregated during the 2 hrs without CO₂, in a manner previously shown to yield chondrocytes (Duke et al., 2011). Alcian Blue staining proves the presence of sulfated polysaccharide in the cartilage matrix.

Conclusion Based on Alcian Blue staining, the cell aggregates in the Silastic membrane are indeed chondrocytes. To prove the validity of this theory, the data from immunohistochemistry test for collagen type II will be needed.

ABSTRACT

HPV typing of Pontential Epidermodysplasia Verruciformis Lesion

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Key Words: HPV, Epidermodysplasia Verruciformis, NMSC

Epidermodysplasia Verruciformis (EV) is a rare genodermatosis causing benign and malignant lesions over the body. Known for its high association with the Human Papilloma Virus (HPV). More specifically, the beta papillomaviruses e.g. type 5, 8, and 14. Half of all these patients develop non melanoma squamous cell carcinomas (NMSC) in sun exposed areas of the body. Additionally, another classification of EV is seen in transplant and immunocompromised patients termed as 'acquired EV'.

DNA was extracted from a paraffin-embedded skin lesion. Polymerase Chain Reaction (PCR) method specific for beta globin reference gene was utilized to asses DNA quality. Nested consensus primer sets were used for amplification of putative viral sequence. After agarose gel electrophoresis, the putative HPV fragments were isolated and cloned with TOPO TA procedure. Nine bacterial colonies were propagated to isolate plasmid vectors containing the HPV fragments. Plasmids were sent for sequencing. The Basic Local Alignment Search Tool (NCBI-Blast) was used to analyze the sequencing results.

The positive beta globin PCR proved amplifiable quality of the extracted DNA. Putative beta HPV fragments were obtained by nested consensus primer sets. After successful cloning and sequencing of the putative HPV PCR products, the NCBI-BLAST search identified the presence of HPV type 5. Considering Beta HPV's have been highly associated to EV disease, presence of infection with HPV type 5 suggests that this patient has EV disease. Additional genetic information of EVER1/EVER 2 mutations may be able to attest further diagnosis.

ABSTRACT

Determining the Role of Lipin1 in Breast Cancer Pathogenesis

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Supported by: Guangwei Du, PhD, Department of Integrative Biology and Pharmacology;
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Key Words: Lipid Metabolism, Lipin1, Breast Cancer

Background: Lipid metabolism is critical for the growth and proliferation of cancer cells. Specifically, two pathways, phospholipid synthesis and fatty acid oxidation, provide cancer cells with the raw materials to spread and the energy to do so. Previous experiments show that the knockdown of Lipin1, a dual-function protein that contributes to both of these pathways, leads to significant cell death, a decrease in cellular growth, and a decrease in proliferation. While this is exciting, the mechanism leading to these results needs to be determined if it is to be utilized to develop novel breast cancer therapies.

Methods: Lipin1 knockdown was accomplished using small hairpin RNAs (shRNAs) in the context of a tetracycline-inducible plasmid system. These plasmids were directly transformed into a HCC1806 cell line along with mutated rescue plasmids generated by PCR amplification. A wild type mutant will fully restore the cell's capacity to synthesize phospholipids and oxidize fatty acids. A phosphatidic acid (PA)-binding deficient mutant will determine Lipin1's ability to bind to PA, a possible signaling molecule and regulator. A transcription-deficient mutant will hamper the cells ability to oxidize fatty acids, but its ability to synthesize phospholipids will be rescued. A PAP-deficient mutant will lead to greatly decreased phospholipid synthesis, but the cells ability to oxidize fatty acids will be rescued. Comparison of cell shape, structure, growth, proliferation, and number among HCC1806 samples with different combinations of inducible and rescue plasmids will yield valuable insight into the mechanism by which Lipin1 knockdown kills breast cancer cells.

Results: The plasmids producing the control, Lipin1 shRNAs, and rescue mutations have been generated. In addition, tet-inducible HCC1806 cell lines expressing shRNA have been generated for future use. Elucidating the deregulation of lipid metabolism by Lipin1 knockdown in breast cancer cells could help to develop a novel therapy for the disease.

ABSTRACT

Identification of potential effector molecules translocated through *Wolbachia* type IV secretion systems during pathogenesis

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Key Words: Type IV secretion system (T4SS), Cre recombinase, *Wolbachia*

Purpose: Type IV secretions systems (T4SSs) are macromolecular complexes responsible for the transport of protein, DNA, and nucleoprotein across the cell envelopes of Gram-negative and Gram-positive bacteria. Initially, I focused on the T4SSs of *Rickettsiales*, a facultative arthropod pathogen closely related to *Legionella* and *Coxiella*, whose type IV apparatus is specifically pivotal in infection. The purpose of my study was to test whether suspected *Wolbachia* (a genus of *Rickettsiales*) effector proteins are translocated across the T4SS. My plans consisted of fusing suspected effector proteins to the Cre recombinase reporter, whose activity would in turn be detected upon transfer to a specific target cell. Because it is not possible to grow *Rickettsial* species in lab culture or genetically transform them, I planned to test for translocation of Cre-effector fusion proteins through T4SSs encoded by *Escherichia coli* and *Agrobacterium tumefaciens*. The reporter strain is *E. coli* with a *lox* cassette introduced into a chloramphenicol acetyl transferase gene. If the donor strain delivers the Cre-effector protein into the reporter strain, Cre excises the *lox* cassette resulting in chloramphenicol resistance. **Methodology:** My main project involved cloning suspected effector genes that were sent to the Christie lab from other labs working on *Wolbachia* and *Rickettsia* species. I introduced restriction sites of interest into the 5' and 3' ends of the effector genes by PCR amplification, then cloned the PCR products into a pGEM-Teasy plasmid. Once I confirmed that the genes were cloned, I subcloned them downstream of the Cre recombinase gene on a plasmid so that the Cre-effector fusion protein is produced upon induction of an arabinose-inducible pBAD promoter. Finally, I planned to introduce the Cre-effector plasmids into *E. coli* cells carrying the conjugative plasmid pKM101 to test for delivery of the Cre fusion protein through the pKM101-encoded T4SS. I also planned to test for translocation through the *A. tumefaciens* VirB/VirD4 system. **Summary of the results:** I was able to clone all of the *Wolbachia* effector genes into the pGEMT-easy vector. I was also successful in subcloning one of the effector genes downstream of the cre gene on a pBAD plasmid. **Conclusion:** My results will aid in identification of possible factors that contribute to the virulence of various *Rickettsial* pathogens of medical importance. Results of my studies will also provide insights into the flexibility of T4SSs in recognition and translocation of substrates from different bacterial species.

ABSTRACT

Investigation of cellular chaining in *Escherichia coli*

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Supported by: William Margolin, PhD, Department of Microbiology and Molecular Genetics ;
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Key Words: bacterial cell division, FtsN, immunofluorescence microscopy

Understanding the process of cell division in microorganisms such as *Escherichia coli* is not only important for our knowledge of how cells divide but also for identifying new antimicrobial targets. Cell division in *E. coli* normally involves the formation of a ring of FtsZ at midcell after the replication and segregation of the nucleoid. The Z-ring then recruits other cell division proteins to midcell and the resulting cell division machinery enables the constriction of the Z-ring along with the cell envelope. The last essential division protein recruited to midcell is FtsN, which interacts with proteins involved in peptidoglycan synthesis and degradation as well as invagination of the outer membrane. It was previously observed that cells overexpressing *ftsN* exhibit a cell division delay and, after many hours of induction, some cells begin to constrict but do not separate, thus forming chains. To investigate the mechanism behind this phenomenon, I recapitulated the phenotype by finding a strain and growth condition that produced many chained cells. I then performed immunofluorescence microscopy to observe Z-rings in the chains. The Z-rings were not located at constriction sites, but were instead located at future division sites between constrictions. Furthermore, when cells overexpressing *ftsN* were plated on minimal media, a loss of cell viability was observed. Together, these data show that overexpression of *ftsN* prevents cell separation in some cells and also decreases cell viability when grown in minimal media. Because overexpression of *ftsN* allows cells to constrict but not fully separate, FtsN may have a role in the final stages of division including daughter cell separation. Further experiments will include isolating suppressors capable of normal growth in minimal media. The location of these mutations may help us identify the essential role of FtsN.

ABSTRACT

Identifying Genetic and Metabolic Components Important for Alkalinization by *Candida albicans*

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Key Words: *Candida albicans*, alkalinization, CAR1, amino acids

Candida albicans is the most common fungal commensal in the human body and can often cause opportunistic infections such as oral thrush and systemic candidiasis. Macrophages are a key component of the innate immune system charged with curbing *C. albicans* proliferation.

Acidification activates degradative enzymes and inhibits hyphal formation. In vitro, in conditions similar to what is within the phagolysosome, *C. albicans* can alkalinize acidic environments; therefore, triggering *Candida* to switch from yeast to filamentous hyphal form. This involves the catabolism of amino acids and the model proposes that basic amine groups drive alkalinization. As an important fungal pathogen in humans, identifying key genetic and metabolic components of the alkalinization process will lead to a better understanding of the fungi's interaction with our innate immune system and potential development of anti-fungal drugs. Through microarray analysis, CAR1, which encodes an arginase, is upregulated during alkalinization along with other amino acid hydrolases and permeases. To determine the role of CAR1 in alkalinization, a *car1* deletion strain was tested in media containing casamino acids as a sole carbon source. The deletion strain alkalinizes and grows at the same rate as the wildtype control, indicating CAR1 does not have an impact on alkalinization. To uncover other genes involved with amino acid import and degradation, a library screen of transcriptional mutants was tested for growth defects in casamino acid media. This screen revealed ten candidate transcription factors that will be confirmed by secondary screening methods in the future. Finally, we hypothesized the amine group of the amino acid is the source of ammonia in alkalinization; however, this was directly tested by comparing alkalinization in the presence of specific amino acids relative to their deaminated cognates. Results indicate the wildtype is able to utilize deaminated compounds resulting in an even greater rate of alkalinization compared to the native amino acid. One possibility to explain these results is the ammonium sulfate, nitrogen source, in the media is generating the ammonia extruded from the cell. Alternative nitrogen sources were tested for growth and environmental alkalinization in wildtype *C. albicans*. All tested alternative nitrogen sources produced similar growth and alkalinization rates. These preliminary results require further confirmation. Though results from the deaminated carbon source experiment were unexpected, the source of the ammonia remains elusive. In addition, CAR1 was not critical for alkalinization; however, several putative transcription factors have been uncovered, which may provide further understanding of this complex process.

ABSTRACT

Bacterial Interaction with Factor H

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Key Words: *Bacillus anthracis*, alternative pathway, factor H, BclA

Bacillus anthracis is a gram-positive bacterium that causes the disease anthrax. There are three types of anthrax infections: inhalational, cutaneous, and gastrointestinal. The spore of *B. anthracis* initiates the anthrax infection and is likely to interact with the host complement system, which is important for host defense against microbial pathogens. The complement system is composed of three pathways: the classical, lectin, and alternative pathways. All three pathways form the important C3 convertase which leads to the cleavage of C3 into C3a and C3b.

Factor H (FH) is a regulator of the alternative pathway and acts as an immune suppressor by accelerating the decay of C3 convertase, playing the role as a cofactor for Factor I mediated cleavage and inactivation of C3b, and by recognizing specific markers, specifically polyanions on host cells. Recent literature has reported that various pathogens utilize FH as a mechanism to evade the host complement system. It is likely that spores of *B. anthracis* also have the ability to recruit factor H to their cell surface. The spores of *B. anthracis* have a single surface protein named Bacillus collagen-like protein of anthracis (BclA). Therefore, we investigated the interaction between BclA and FH and characterized the binding mechanism between them. First we purified a his-tagged recombinant protein of the C-terminal domain of BclA (BclA-CTD) by nickel chromatography and used ELISA-based assays to characterize BclA and FH interaction. The results indicate that the BclA-CTD can directly bind to FH and this interaction was most favorable under low salt concentrations. To localize the region on FH that BclA is likely to target, we used two known ligands of FH, heparin and C3b, to test if BclA-FH have overlapping binding sites. We found that heparin inhibited BclA-CTD binding to FH in a dose-dependent manner, but C3b had no significant effect on the interaction. These studies are likely to reveal a novel immune suppression mechanism by *B. anthracis*.

ABSTRACT

Peptide Adjuvants Increase Processing of BCG Vaccine in Antigen Presenting Cells

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Key Words: Tuberculosis, Antigen Presenting Cells, TLR-2 ligands, Vaccine, Macrophage

Tuberculosis due to *Mycobacterium tuberculosis* (Mtb) is the leading cause of death due to curable infectious diseases. A preventative vaccine could save millions of lives each year, but the current vaccine, Bacillus Calmette–Guérin (BCG), is less effective against adult TB. *Hypothesis:* Macrophages and dendritic cells (Antigen Presenting Cells; APCs) process BCG antigen and activate CD4 T cells through the MHC-II pathway, which under the influence of Th1 cytokines protect against tuberculosis. However, BCG hides in an immature phagosome of macrophages without fusing with lysosomes, which prevents its efficient processing. BCG also induces less strong Th1 cytokines IL-12, IL-1 β , and TNF- α . We therefore hypothesized that a peptide adjuvant from the CFP10 protein endogenous to Mtb could enhance BCG efficacy. In earlier studies, the peptide fragment ‘C5’, a TLR-2 activating ligand, was added with BCG to mice, and BCG was more efficiently processed enhancing the immune protection against tuberculosis. *Methods:* In this study, the peptide region around C5 was broken up into four smaller peptides, which were re-synthesized to find where most of C5’s adjuvant-like activity is located, and to find which of the four gave the strongest immune response. APCs cultured from the bone marrows of mice were activated with each of the peptides, infected with BCG, and overlaid with T cells specific for Antigen 85B. Using a sandwich ELISA, the amounts of the Th1-activating cytokines IL-2, IL-12, IL-1 β , and TNF- α released by the APCs and T cells in the supernatants were analyzed. *Results and Conclusions:* Compared to un-activated but BCG-infected APCs, all four of the peptides induced greater levels (approximately 2-fold) of IL-2 from T cells. However, the peptides differed in their ability to induce Th1 cytokines. IL-1 β levels were only significantly raised by APCs treated with C5₁ and C5₂. Likewise, IL-12 levels were increased by C5₁, C5₂ and C5₃. However, TNF- α levels were not significantly affected by all four peptides. Because IL-12 and TNF- α are involved in T-cell pathways that cause rapid, robust, yet short-lived, responses to infection, we suggest that the first three peptides of the originally identified C5 are the major adjuvant segments. Interestingly the first two segments also induce IL-1 β which is involved in a different T-cell pathway enhancing long-lasting memory T-cells. A good vaccine must induce both short-term immunity and long-term memory. We have now determined that the first three segments of C5 peptides have multiple adjuvant activities contained within and these domains are sufficient to induce a strong adjuvant activity enhancing the immune response to BCG.

ABSTRACT

Quantitation of Pathological Changes in the Ascending Aorta

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Supported by: Dianna M. Milewicz, MD, PhD, Department of Internal Medicine

Key Words: Thoracic Aortic Aneurysm, matrix metalloproteinase, ACTA2 gene

Introduction: Thoracic aortic aneurysms (TAAD) leading to acute ascending aortic dissection and rupture are one of the common causes of premature deaths. Up to 20% of individuals who present with TAAD have first-degree relatives similarly affected, termed familial TAAD (FTAAD). Specifically, missense mutations in α -actin (ACTA2 gene) are responsible for 14% of TAAD and dissections. Histologic analysis of patients affected demonstrates aberrant thickening of the aortic media with fragmentation and disarray of elastic fibers. Ultimately, the specific pathology of FTAAD is not entirely known.

General Methods: To investigate pathological changes due to TAAD, we conducted qPCR assays on Acta2^{-/-} mouse models to examine the gene expression of angiotensin II type I receptor, CYBA, metalloproteinase, angiotensin converting enzymes, in which we expected an increase of expression. Furthermore, we used colorimetric analysis to look at the pathological changes in the ascending aorta. Utilizing Image J, we were able to determine the ratio of elastin pixels versus lamellae pixels, allowing us to evaluate elastin fiber integrity and degradation, which is a marker of aneurysm progression. This method was tested on several systems elucidating the pathology of TAAD: 1) the effects of doxycycline, a non-specific inhibitor of mmps, on elastin degradation in Acta2^{-/-} mice, 2) degree of elastin degradation over time in Acta2^{TG R258C} mice 3) the effects of Myh11^{R247C} as a modifier mutation for aortic disease and when crossed with Acta2^{-/-} mice.

Conclusions: Aneurysm pathology in mice can be objectively and quantitatively characterized by colorimetric analysis using pixel quantitation of elastin percentage. As a result, the Acta2^{TG R258C} mice displayed a decrease in elastin thickness over time. This suggests that there is indeed elastin degradation due to the Acta2^{TG R258C} mutation. Furthermore, we hypothesized that doxycycline would ameliorate genetically induced TAAD by attenuating elastin fiber degradation, but have yet to find conclusive data. Nevertheless, this study illustrates the promise that enhanced identification of drugs in this research stage will have pronounced effects on medicine. Interrogating pathogenesis and deriving unanticipated disease mechanisms is but an obligate final step before creating rational treatment strategies and beginning clinical trials on individuals afflicted by TAAD.

ABSTRACT

Role of PPAR γ in Glutamine's Mitigation of Distant Organ Injury During Intestinal Ischemia-Reperfusion

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Class of 2013

Sponsored by: Rosemary Kozar, MD, PhD, Department of Surgery

Supported by: Rosemary Kozar, MD, PhD, Department of Surgery; University of Texas at Houston Medical School - Office of the Dean

Key Words: PPAR γ , Glutamine, Ischemia-Reperfusion, Lung

INTRODUCTION: Previous studies have found that pro-inflammatory and injurious factors carried in trauma-hemorrhagic shock (T/HS) mesenteric lymph can induce distant organ injury. This suggests that mitigating intestinal injury following T/HS may also lessen lung injury, the most frequently injured organ after T/HS. We have previously demonstrated that enteral glutamine activates peroxisome proliferator-activated receptor- γ (PPAR γ) and is linked to a decrease in gut mucosal injury and inflammation. **AIM:** We therefore hypothesized that enteral glutamine functions via the intestinal PPAR γ nuclear receptor to abate injury in distant organs following mesenteric ischemia-reperfusion. **METHODS:** Superior mesenteric artery occlusion, a well-characterized model of intestinal ischemia/reperfusion (I/R), was applied to wild type (WT) and PPAR γ knockout (KO) mice. Mice underwent intestinal ischemia for one hour then \pm glutamine (60mM via enteral sac) at the time of reperfusion and were compared to shams. After six hours, lungs were harvested and analyzed. The wet/dry ratio was assessed to measure pulmonary edema. Histopathologic injury was scored using a three-point scale based on alveolar thickness, capillary congestion, and cellularity. Finally, inflammation was measured through quantification of myeloperoxidase (MPO) immunostaining. **RESULTS:** WT IR mice demonstrated wet/dry ratios and histopathologic scores significantly higher than for the IR KO mice. Furthermore, lung MPO immunostaining was found to be stronger in the KO mice than in the WT mice. Glutamine mitigated lung edema, injury, and inflammation in WT but not KO mice after IR. **CONCLUSION:** This data suggests that the PPAR γ nuclear receptor has a vital role in glutamine's mitigation of distant organ injury.

ABSTRACT

Assessing the effectiveness of Androgen Deprivation Therapy plus Chemotherapy in Hormone Naïve Prostate Cancer Patients

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Sponsored by: Robert Amato, DO, Department of Oncology

Supported by: Robert Amato, DO, Department of Oncology*; University of Texas at Houston Medical School – Office of the Dean*

Key Words: prostate cancer, androgen deprivation, chemotherapy

Background: Prostate cancer is the second leading cause of cancer-related deaths in the United States. 241,740 men are expected to be diagnosed with prostate cancer in 2012 and 28,710 of these men are predicted to die from it. Increasing serum levels of prostate-specific antigen (PSA) post local therapy (prostatectomy, radiation, or both) are managed with androgen deprivation. It is also the first-line treatment in patients ineligible for local therapy. Androgen deprivation is palliative and adversely affects quality of life and overall health (eg. decreased libido, weight gain, hot flashes, bone density loss). For castration-resistant patients, chemotherapy is often the next-line treatment. Chemotherapy is associated with increased overall survival. This study examines the efficacy of androgen deprivation plus chemotherapy as initial treatment for patients who are ineligible for local treatment or have failed local therapy. Our purpose is to correlate the application of chemotherapy plus androgen deprivation (chemohormones) to androgen-dependent and independent prostate cancer cells with a longer duration of undetectable PSA, progression free survival and overall survival.

Methods: Patients received androgen deprivation in the form of leuprolide every twelve weeks and bicalutamide after the completion of chemotherapy. One cycle consists of ketoconazole and doxorubicin on weeks 1, 3, and 5, estramustine and docetaxel on weeks 2, 4, and 6, and no treatment on weeks 7 and 8 to allow for recovery. Patients received 3 cycles of chemotherapy with 12 months of androgen deprivation, 4 cycles of chemotherapy with 18 months of androgen deprivation, or 5 cycles of chemotherapy with 24 months of androgen deprivation. In this retrospective study, 71 patients were identified with biopsy confirmed prostate carcinoma. Baseline PSA, pathology, initial radiographic presentation, patient history, tumor-related biomarkers, and post-treatment outcomes were collected. Patients who received 3 cycles of chemotherapy presented with PSA rise only; patients who received 4 cycles of chemotherapy presented with PSA rise and minimal radiographic evidence of disease; patients who received 5 cycles of chemotherapy did not receive local therapy and presented with greater tumor burden.

Results:

# of Chemotherapy cycles	3	4	5	Currently receiving treatment
# of Patients	43	9	5	14
Baseline PSA (median, range)	1.2, <0.1-163.1	14.5, 0.8-120	172.2, 76.4-1961	2.7, 0.6-427.1
PSA post chemotherapy (median, range)	<0.1, <0.1-301.1	0.1, <0.1-2.9	1.43, 0.2-3.4	Still Active
PSA post androgen deprivation therapy (median, range)	<0.1, <0.1-1257	1.45, <0.1-16.9	Still Active	Still Active

Conclusion: Chemotherapy plus androgen deprivation demonstrated clinical benefit in all subsets of patients. The patients who received 3 cycles of chemotherapy had the greatest treatment response, as predicted for this patient population based on initial presentation of disease. Patients who received 5 cycles of chemotherapy had least treatment response as expected due to greater tumor burden at presentation.

ABSTRACT

Genetics of Ankylosing Spondylitis

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Supported by: Xiaodong Zhou, MD, MS, Department of Rheumatology; University of Texas at Houston Medical School - Office of the Dean

Key Words: Ankylosing spondylitis, HLA-B27, ERAP1, IL1R, IL23R, IL12B, IL-17-IL-23 pathway, NF- κ B pathway, TBKBP1, TRADD, CARD9

Ankylosing spondylitis (AS) is a chronic inflammatory arthritis of the axial skeleton involving inflammation of the spine and extra-spinal sites that lead to limited movement of the peripheral joints and non-articular structures. It appears more in families, especially in monozygotic twins, which have been reported with high concordant rates, indicating the crucial role of genetic contributions to the disease. Recently, extensive genetic studies through the candidate gene approach and Genome-Wide Associated Studies (GWAS) have revealed multiple genes within the MHC locus such as HLA-B27, the first gene discovered for AS heritability, genes outside the MHC region such as ERAP1, an aminopeptidase gene, IL1R, IL23R and IL12B, cytokine genes involved in the IL-17-IL-23 pathway, and TBKBP1, TRADD and CARD9, cytokine-producing genes involved in the NF- κ B pathway. The latter three genes have functions overlapping the IL-23 and NF- κ B pathways through Th cell differentiation and NF- κ B activation, thus suggesting that these genes, and possibly others yet discovered may be the factors that connect both pathways to AS pathogenesis. This review summarizes the candidate AS-associated genes reported thus far, categorizes the pathway-related candidate genes, and discusses the potential biological influences.

ABSTRACT

Potential progenitor cells for oligodendrocytes, astrocytes, and neurons: A previously neglected cell population in rat microglia cultures

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Sponsored by: Jaroslaw Aronowski, MD, PhD; Xiurong Zhao, MD, Department of Neurology
Supported by: Jaroslaw Aronowski, MD, PhD; Xiurong Zhao, MD, Department of Neurology;
University of Texas at Houston Medical School – Office of the Dean
Key Words: microglia, brain progenitor cells, neurons, oligodendrocytes, astrocytes

Introduction. Brain microglia in culture have previously been reported to be a potential source (progeny) for other brain cells (e.g. oligodendrocytes, neurons, and astrocytes). However, it is not clear whether these newborn cells are derived from de-differentiated microglia or are from co-existing (co-purified) progenitor cells. We identified a unique cell population in the microglia culture, which were suspended in culture media but also loosely attached to fully attached cells. We hypothesized that these cells may possess the capacity to differentiate into other brain cells.

Methods and Results. Using immunohistochemistry, we found the purified microglia cultured from prenatal rat brains to be almost 100% CD11b+ or CD68+ (microglia markers) on the first day after seeding cells onto TC plates. Using Western blot and RT-PCR analysis, we found that these attached microglia cells did not express markers for other brain cell types (e.g. neurofilament, MBP). However, as the culture time span increased, the cells' morphology and phenotype gradually changed. We demonstrated that over time, the majority of cells began to express markers for immature and mature neurons (e.g. nestin, DCX, Tuj1, MAP2, neurofilament), oligodendrocytes (nestin, O4, OSP, MBP) and astrocytes (GFAP, vimentin). Concurrently, microglia markers gradually reduced in number during this culture period. Upon closer examination of microglia in cultures, we noted an interesting phenomenon: a distinctive cell population floating in the medium of the microglia culture. These cells were loosely attached by strings of cells to other cells that were fully attached to the plate. These were previously neglected and most likely removed during media changes. This population was BrdU+, Ki-67+, and PCNA+, implying that these cells were proliferating cells. By double immunohistochemistry, we showed that this population of cells expressed markers for progenitor or newborn/immature brain cells (e.g. nestin, DCX, Tuj1, O4 and vimentin).

Conclusion. We propose that these suspended but loosely attached cells in the primary microglia culture are pluripotent and serve as a source for other brain cells, including neurons, oligodendrocytes and astrocytes.

International Medical Students

ABSTRACT

3D structures of Lyme disease spirochete revealed by Cryo-electron tomography

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Class of 2018

Sponsored by: Jun Liu, PhD, Department of Pathology and Laboratory Medicine

Key Words: *Borrelia burgdorferi*, Infectious diseases, Lyme disease, Spirochete, Cryo-Electron

Borrelia burgdorferi belongs to a group of bacteria, called spirochetes, which are notorious for several infectious diseases such as syphilis, leptospirosis and Lyme disease. In particular, Lyme disease is the most common vector-borne infection in the United States, and has shown a steady increase in incidence since its discovery in 1975. The disease is caused by *B. burgdorferi* and other closely related spirochetes transmitted to humans via infected *Ixodes* ticks. *B. burgdorferi* is a motile spirochete with distinct flat-wave morphology. Bundles of periplasmic flagella, which lie between inner and outer membrane, are mainly responsible for the unique bacterial shape. The motility of *B. burgdorferi* results from coordinated rotation of the flagellar motor at both tips of the cell. Of the ~50 genes involved in the expression and assembly of the flagellum, *motA* and *motB* are two genes essential for torque generation, yet their impact on morphology and infectivity is unknown. In this study, our collaborators constructed a non-polar *motB* deletion mutant, which is non-motile and non-infectious. Moreover, the cell shape doesn't remain flat-wave and is conspicuously elongated. We reconstructed the three-dimensional (3-D) structures of intact *B. burgdorferi* cell from wild-type (WT), and *motB* mutants by utilizing a cut-edge imaging technique, cryo-electron tomography. We discovered that parts of the flagella are not as tidy as wild type, while some parts still form a flat-ribbon surrounding the cell body in *motB* cells as those seen in WT cells. Complementation *in trans* (*motB*⁺) restored the synthesis of MotB, the flat-wave morphology, the motility and the infectivity. Therefore, we conclude that the MotA/MotB complex is the torque generator that is essential for motility, morphology, and infectivity of *B. burgdorferi*.

ABSTRACT

Reviving the original McGurk effect in an fMRI study

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Sponsored by: Michael S. Beauchamp, PhD, Department of Neurobiology and Anatomy

Key Words: fMRI, McGurk effect, Audiovisual Integration, Speech Perception

Introduction

In everyday conversation, we are presented with both auditory (vocal sounds) and visual information (mouth movements). Integrating both pieces of information allows us to more accurately understand speech. The McGurk effect is an audiovisual speech illusion in which the auditory input ("ba") and visual input ("ga") are mismatched, creating an entirely new percept ("da") (McGurk & MacDonald, 1976). The original discovery was actually a mistake: a male speaker was filmed, and the vocal sound of syllables were recorded to audiotape. Then, they were manually cut and different auditory and visual syllables were accidentally spliced together. The purpose of this project was to restore the stimuli presented in the original paper so that the original findings could be replicated and so that the original stimuli could be used in an fMRI study, a technique not available at the time of the original study.

Methods

A VHS videotape of the original stimuli was obtained. The analog video was first converted into a digital file using a commercial service (CostCo). The video consisted of one long sequence of 28 short clips spliced together (Table.1). There were two McGurk syllables, auditory "ba-ba" and visual "ga-ga" (illusory percept "da-da") and auditory "pa-pa" + visual "ga-ga" (illusory percept "ta-ta"). Additionally there were several single syllables with the auditory syllable "ba" dubbed onto several different visual syllables. There was also one sentence which also utilizes the McGurk effect with dubbed mismatch words: auditory "My Bad Pought Me to Brive" with visual "My Gad Kought Me to Grive" which results in the illusory percept " My Dad Tought Me To Drive". The total length of this video was 3 min 31sec. From this long video file, separate files were created for each stimulus video: 8 double syllable utterances (approximately 2 sec / each), 16 single syllable utterances (approximately 2 sec / each) and 2 long sentences (approximately from 4 to 5 sec/ each). These clips were named such that the stimulus title (ex: "AbaVga") explained what the auditory utterance (A) and the visual utterance (V) were for each clip. There are three kinds of stimuli: congruent stimuli e.g. Aba + Vba, shown in the table in incongruent stimuli e.g. Aba + Vsha (percept "ba"; shown in table as underlined); McGurk incongruent stimuli e.g. Aba + Vga (percept "da", shown in table as bold).

Table.1 Contents of original McGurk stimulus video.

<i>audio</i>	<i>video</i>	<i>percept</i>
<u>ka-ka</u>	<u>ba-ba</u>	<u>ka-ka</u>
ba-ba	ga-ga	da-da
pa-pa	ka-ka	ta-ta
<u>ga-ga</u>	<u>ba-ba</u>	<u>ga-ga</u>
<u>ga-ga</u>	<u>pa-pa</u>	<u>ga-ga</u>
<u>ba-ba</u>	<u>ka-ka</u>	<u>ba-ba</u>
<u>pa-pa</u>	<u>ga-ga</u>	<u>pa-pa</u>
<u>ka-ka</u>	<u>pa-pa</u>	<u>ka-ka</u>
<u>ba</u>	<u>pa</u>	<u>ba</u>
<u>ba</u>	<u>va</u>	<u>ba</u>
<u>ba</u>	<u>tha</u>	<u>ba</u>
<u>ba</u>	<u>da</u>	<u>ba</u>
<u>ba</u>	<u>ta</u>	<u>ba</u>
<u>ba</u>	<u>sha</u>	<u>ba</u>
<u>ba</u>	<u>la</u>	<u>ba</u>
<u>ba</u>	<u>ha</u>	<u>ba</u>
<u>ba</u>	<u>ka</u>	<u>ba</u>
<u>ba</u>	<u>na</u>	<u>ba</u>
<u>ba</u>	<u>cha</u>	<u>ba</u>
<u>ba</u>	<u>sa</u>	<u>ba</u>
ba	ga	da
<u>ba</u>	<u>dha</u>	<u>ba</u>
<u>ba</u>	<u>fa</u>	<u>ba</u>
ba	ba	ba
My Bad Pught Me to Brive	-	My Bad Pugh Me to Brive
My Bad Pught Me to Brive	My God Kought Me to Grive	My Dad Tought Me to Drive

The sound in the original was of very poor quality and contained a great deal of noise. Worse, for some videos the lip movements and the auditory words were asynchronous. To improve the quality, the stimuli were edited. First, by using Final Cut Pro (Software in Mac), the visual component and the audio component were separated. Then, in order to adjust the gap between the starting of sound and mouth opening, the start of sound was reset so that the sound starting and mouth opening were synchronous. In addition, two types of audio filter were applied the audio. One was the audio filter in Final Cut Pro. The other was the noise removal effect (parameters: 24 dB noise reduction, 150 Hz frequency smoothing and 0.15 secs Attac / decay time) in Audacity. The Audacity filter gave better results, and increased the signal to noise ratio so that each syllable was more easily understood. A small strip at the bottom of the video frame contained random noise distortion and was removed by applying the crop effect in Final Cut Pro. Some of the video clips were dark, so by applying color balance effect, the brightness was adjusted. Finally, to make the volume of all the clips equal, the volume of each clip was adjusted in Final Cut Pro.

Results

To compare our results with those reported in McGurk & Macdonald (1976) we presented 10 repetitions of each of four different video clips (Ababa+Vgaga, Agaga+Vbaba, Akaka+Vpapa, Apapa+Vkaka) to 5 English speakers. The clips were presented in random order. In the original McGurk paper, subjects perceived the McGurk effect 98% of the time. In our replication, 52% of subjects perceived the McGurk effect with the “Ababa+Vgaga” stimulus (percept “da-da”) and 38% of subjects perceived the McGurk effect with the “Apapa+Vkaka” stimulus (percept “ta-ta”).

Conclusion

The videotape of the original McGurk stimulus was very poor quality. It was noisy, dark, and the voice and video were not synchronized. All of the stimuli were in one film, so it was very hard to access each stimulus clip. For this project, this video was restored in digital clear short clips. We replicated the findings of the original McGurk study using the original stimuli, showing that mismatched auditory and visual syllables can create the percept of an entirely different syllable. McGurk susceptibility in our replication was lower than in the original study. This may be because of the difficulty in removing auditory noise during the utterances (as opposed to removing noise in the silent periods between utterance). If the auditory voice was hard to hear, this could reduce McGurk susceptibility. Supporting this idea, modern McGurk stimuli recorded with digital video and low noise produces higher rates of susceptibility. However, having the original McGurk stimuli available to researchers represents a valuable shared resource. These clips will be uploaded to YouTube allowing free access by researchers anywhere in the world. In future experiments, we will use fMRI to examine brain activity during the McGurk effect with the original McGurk stimuli.

ABSTRACT

Production of Müller Glia is Affected by RGC Shortage

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Class of 2017

Sponsored by: Steven W. Wang, PhD; Department of Ophthalmology & Visual Science

Key Words: Math5, Brn3b, RGC, Müller glia, retinogenesis, retina, mouse

Background: The vertebrate retina contains six major neuronal and one glial cell types that are derived from a common progenitor pool. Among all retinal cell types, retinal ganglion cells (RGCs) are the first to differentiate. It is believed that RGCs play important roles in retinogenesis since they are required for producing a normal number of retinal cells. Here, we study the influence of RGCs on Müller glia (MG) production by examining the number of MG in various RGC deficient environments. **Methods:** Three different knockout mice with varying degrees of RGC loss were generated: *Brn3b*^{-/-} has 80% RGCs loss, *Math5*^{-/-} has 95% RGCs loss, and *Brn3b*^{-/-}; *Math5*^{-/-} has ~99% RGCs loss. Wildtype retinas also included as controls. Eyeballs from these 4 genotypes at the stage of P5, P10, P15, P21 and P30 were collected and fixed in 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) on ice, then infiltrated in 20% sucrose overnight after 3-times washing with PBS, and embedded in O.C.T. Radial cryosections were collected at 30µm thickness. Two most center sections in each retina were used for immunolabeling. Sections were incubated with the Primary antibody Sox9 overnight at 4°C. Samples were equilibrated to room temperature for 1 hour next morning, followed by incubation with secondary antibody, Alexa488 goat anti rabbit IgG. Propidium iodide (PI) was used to stain nuclei after treating with RNaseA. Images were digitally captured using a Zeiss 510 confocal microscope. Cell counting was proceeded by naked eyes with the assistance of a Tally Counter. **Results:** Data collection remains incomplete. However, the preliminary results show production of Sox9-positive cells reduced in all RGC-depleted retinas with one exceptions that *Math5/Brn3b*-double deficient retina has more Sox9-positive cells then the wildtype at P5. Results are consistent with previous independent study in the lab. **Conclusion:** Production of MGs is lessened due to RGC shortage.

ABSTRACT

The requirement of FtsA ATP binding for *E. coli*'s cell division

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Class of 2017

Sponsored by: William Margolin, PhD, Department of Microbiology & Molecular Genetics

Key Words: FtsZ, FtsA, IFM

FtsZ, FtsA, and ZipA are proteins essential to cell division in most prokaryotes. Our lab studies the molecular process of cell division in the bacterium, *Escherichia coli*. When *E. coli* starts binary fission, FtsZ will move to the middle of the rod-like cell and form the Z-ring. As soon as the Z-ring forms, it triggers the downstream protein FtsA to localize to the center of the rod-like cell. FtsA and ZipA contribute to other cell division proteins localizing sequentially to the Z-ring. Once all of the cell division proteins localize to the mid-cell, the Z-ring is functional. We call this macromolecular machine the “divisome”. If FtsZ or FtsA acts abnormally or becomes non-functional, the cell will stop dividing and become filamentous. Our project this summer was focused on the protein FtsA. We tried to understand how ATP contributes to the function of FtsA.

To answer this question, we devised several experiments for the engineered strain, WM4111, (MG1655::ftsA27/pDSW210-FLAG-ftsA27), which produced a mutated protein, FtsA27. FtsA27 is a mutant of FtsA with a point mutation that is located at the ATP-binding site. We know that cells expressing FtsA27 can grow normally at 30°C, but become filamentous at 42°C. We hypothesized that FtsA27 fails to localize to the middle of the cell and recruit downstream proteins to the Z-ring at 42°C.

To test our hypothesis, we visualized FtsA27 localization at 30°C and 42°C using immunofluorescence microscopy (IFM). We used rabbit polyclonal anti-FtsZ (Z44) antibody and monoclonal anti-FLAG antibody (M2) as the primary antibodies; goat anti-rabbit antibody, conjugated to a green fluorescent molecule (Alexa 488) and goat anti-mouse antibody, conjugated to a red fluorescent molecule (rhodamine), as the secondary antibodies.

Results: We got clear bands at mid-cell from WM4111 cells with anti-FLAG and anti-FtsZ at 30°C; . no bands on anti-FLAG images but clear bands on anti-FtsZ images at 42°C. This indicates that FtsA27 localizes to Z-rings at 30°C but not at 42°C.

These results support the idea that ATP may play a crucial role in FtsA's binding to the Z-ring.

ABSTRACT

Protection of IL-27 against Brain Ischemic Stroke *in vivo* and *in vitro* in rodents

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Sponsored by: Jaroslaw Aronowski, MD, PhD, Department of Neurology

Key Words:

-27 is a cytokine primarily produced by activated dendritic cells and macrophages in response to Toll-like receptor ligands and pro-inflammatory cytokines. Although it is reported that brain microglia produce IL-27, the effect of IL-27 in brain after ischemic stroke is unknown.

To understand the role of IL-27 in ischemic brain, twelve C57/BJ6 mice (male, 8~10 weeks-old) were subjected to 60mins of MCA/CCA occlusion, a cortical ischemic stroke model, then six were treated with 50 ng/kg of IL-27, sc, at time of reperfusion and then at 24 hrs after ischemia, the other six were used for vehicle control. At 48h after reperfusion, the neurological functional deficit scores (NDS) were measured with a cluster of behavior tests (postural reflex, forward placing, foot fault, cylinder and wire test) and brain edema was determined by quantifying brain water content [(wet weight-dry weight)/wet weight x 100%]. The neurological functional impairment and brain edema in IL-27 treated group was 31.1% and 2.9% less severe than the control, respectively; both measurements were significantly superior to the control ($p \leq 0.05$).

To further identify the expression pattern of IL-27 in brain cells, the primary rat brain cortical cells were then cultured from the E-18 embryos and mRNA expressions were measured using RT-PCR. We found that IL-27 was exclusively expressed in rat microglia but not in other brain cells (neurons, astrocytes or oligodendrocyte); and temporal exposure of microglia to oxygen-glucose-deprivation (OGD, an *in vitro* model of ischemia) promoted IL-27 mRNA induction. Conversely, the IL-27 receptor, named WSX-1/TCCR (also known as IL-27Ra), was expressed in neurons but not in microglia. In response to temporal OGD, the expression of IL-27Ra in neurons was significantly increased.

To understand the effect of IL-27 on neurons in responding to OGD, exogenous IL-27 was delivered to the neurons, and then the neurons were exposed to 60mins of OGD. At 24hrs after reperfusion, the LDH and MTT levels were determined. IL-27 displayed a dose-dependent neuroprotection at 0.001~2.0 ng/ml.

ABSTRACT

Lactoferrin Protects Rat Neurons from Ischemic Injury and Increases Phagocytotic Function of Microglia *in vitro*

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Class of 2016

Sponsored by: Jaroslaw Aronowski, MD, PhD; Xiurong Zhao, MD, Department of Neurology

Key Words: Lactoferrin, neuron, microglia

Background: Lactoferrin (LF) is an abundant iron-binding glycoprotein normally present in bodily fluids. It is synthesized and stored primarily in neutrophils, and neutrophil degranulation is believed to be the main source of circulating LF. Although LF is reported to be present in the brain tissue, it is not clear what the specific source is and what role it plays. **Methods and Results:** To determine the LF's origin in the brain, we used rat primary cell cultures for different brain cell types (neurons, astrocyte, oligodendrocyte, and microglia) and utilized RT-PCR and mRNA extracted from these cells; we found that LF is mainly expressed by neurons and astrocytes. Using co-localization staining (double immunostaining with anti-MAP2 and LF antibodies), we indeed localized LF to neurons. Next, we found that LF protein is present in the neuronal culture media, meaning that LF can be secreted by neurons. When cultured neurons were exposed to oxygen-glucose-deprivation (OGD- an *in vitro* model of ischemia), we found that LF mRNA expression is increased in response to OGD. To study the relevance of LF to OGD-induced neuron injury, we exposed neurons to 2h of OGD in the presence of 0.05~100 μ g/mL LF in the culture medium. We found that LF robustly reduced neuronal damage when measured at 18h after OGD. 1 μ g/mL LF was most effective and reduced LDH by 42.3%. Although it was earlier suggested that microglia could produce LF, under experimental conditions of present study, we did not detect LF mRNA expression by primary rat microglial cells. However, we found that LRP1, the receptor for LF is expressed by microglia and that its expression was strongly induced upon exposing microglia to OGD. Since LF is expressed and released in response to OGD, an important question to ask is: can LF signal to microglia through LRP1 and modify microglia function? To address this, we tested if LF can affect erythrocyte phagocytosis. Indeed, we found that 1~100 μ g/mL LF under normoxic and especially hypoxic conditions could improve erythrocyte uptake by microglia. Using 50 μ g/mL LF the phagocytosis index (number of erythrocytes per microglia) increased 2.3 folds vs. PBS control. **Conclusion:** LF can be synthesized and released by neurons and astrocytes and its synthesis is increased by OGD. Through a wide range of doses, LF is neuroprotective in OGD-injury model. Also, under hypoxic conditions microglia upregulate LF receptor, LRP1, thus, allows neuron-derived LF to stimulate phagocytosis of erythrocytes (a model of hemorrhagic stroke).

ABSTRACT

The effect of AHCC in improving bone mineral density

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Class of 2013

Sponsored by: Anil Kulkarni, MSc, PhD, Department of Surgery

Key Words: AHCC,BMD

During this Summer Research Program period, for my lab project I was assigned to perform data analysis of the work completed earlier. Abstract is given below.

Background: The active hexose correlated compound (AHCC) an alpha-glucan rich nutritional supplement produced from the mycelia of shiitake (*Lentinula edodes*) of the basidiomycetes family of mushrooms. Anti-orthostatic hindlimb weight unloading (HU) of rodent causes immune dysfunction and has adverse physiologic effects associated with space flights, which shares common features with bed rest subjects. It can serve as an accelerated aging model for age related system function decline on the body, effects such as immune dysfunction and antioxidant level decline in aging and now extending to skeletal studies with bone system. The purpose of this study is to explore whether an AHCC supplement is effective for improving bone mineral density.

Methods: 4 to 6 mice were subjected to HU for one week only during which they were fed control chow and AHCC supplemented chow in both HU and non-HU groups. Femur bones were removed for bone mineral density determinations.

Result: Bone Mineral Density (BMD) decreased by 5.9% in the HU control diet (Ci) group compared to the non-HU control (C) group. There was no significant difference in bone mineral density (BMD) between the non-HU control diet (C) group and the non-HU AHCC (A) group. The HU AHCC (Ai) group had decreased BMD when compared to HU control (C) group (BMD -5.9%).

Conclusion: There was no significant difference in the treated and non-treated groups subjected to microgravity for the effect of AHCC in improving bone mineral density. However, more data and experiments are needed to confirm our hypothesis.

In addition, I had also opportunity to observe robotic surgical procedures with Dr. Snyder from Surgery department and rotate in other surgical research labs.

ABSTRACT

Identification of VCP/p97 Ubiquitin Substrates

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Class of 2017

Sponsored by: Ju-Mei Li, PhD, Jianping Jin, PhD, Department of Biochemistry and Molecular Biology

Key Words: Ubiquitin, VCP/p97, TXNIP, PRKAR1A

VCP (valosin-containing protein, also called p97) is an AAA type ATPase that participates in ERAD, and cellular protein ubiquitin and degradation. VCP/p97 is found to associate with ubiquitinated proteins and facilitate their delivery to the proteasome for degradation. When VCP/p97 is silenced, the proteasome-mediated protein degradation was compromised and more ubiquitin-conjugated proteins thus accumulated. To identify those potential VCP/p97 target proteins, the ubiquitinated proteins were enriched by affinity purification and identified by mass spectrometry. Hundreds of candidate proteins were identified and eight of them were studied here.

All eight genes were cloned into the pENTR vector and sequence-confirmed. HA-tagged fusion cDNAs were created using the Gateway LR Clonase. The individual proteins were expressed in HeLa cells and the cells were treated with EerI, a VCP/p97 inhibitor, for 2 hours. Collected cell lysates were resolved on a SDS-PAGE gel and detected using western blotting. We found that ubiquitin-conjugated TXNIP and PRKAR1A were accumulated upon VCP/p97 was inhibited by EerI, suggesting that TXNIP and PRKAR1A are two novel VCP substrates.

TXNIP is originally identified as thioredoxin-interacting protein. In addition to function as oxidative stress mediator, TXNIP is insulin-inducible and can regulate glucose uptake. PRKAR1A encodes the regulatory subunit of cAMP-dependent protein kinase. With cAMP binding, the regulatory subunits dissociate from the inactive holoenzyme to free the active catalytic subunits. PRKAR1A mutation causes Carney complex, which is a multiple neoplasia syndrome. The follow-up study would focus on how the dysregulated degradation and ubiquitin modification would impact on the biological functions of these proteins.

ABSTRACT

ADAM15 regulates syndecan-1 shedding in intestinal epithelial cells

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Sponsored by: Rosemary Kozar, MD, PhD; Zhanglong Peng, MD, PhD; Wei Lin, PhD,
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Key Words: Syndecan-1, ADAM-15, APMA

Background Syndecan-1 is a cell surface heparin sulfate proteoglycan that plays a key role in a wide range of physiological processes, including repair of the intestinal epithelium. ADAMs are a family of ectodomain sheddases which release a variety of cell surface proteins, including growth factors, cytokines, cell adhesion molecules and receptors. ADAM-15 has an integrin-binding motif which involves in cell-cell interactions and it can inhibit repair in intestinal epithelial cells. ADAM-15 may be activated by APMA (p-aminophenylmercuric acetate), a well-known metalloproteases activator. *Hypothesis:* We hypothesized that ADAM15 may inhibit intestinal epithelial repair through regulating syndecan-1 shedding. *Experimental Design:* IEC-6 cells were treated with 0, 1, 2, 4, 8, 16 μ M APMA in conditioned media (DMEM) for 30 minutes. After media collection, cells were harvested for expression of ADAM15 by western blot and syndecan-1 shedding by dot blot. *Results:* Expression of ADAM15 increased in APMA-treated cells compared to controls. Additionally, shedding of syndecan-1 increased in a concentration-dependent manner. *Conclusions* ADAM15 may impair repair of the injured intestinal epithelium by up-regulating syndecan-1 shedding in intestinal epithelial cells.

ABSTRACT

Evaluation of Pool B as primary antibody against GST-NSD1 for Western Blot and Immunoprecipitation

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Class of 2013

Sponsored by: Philip B. Carpenter, PhD, Department of Biochemistry and Molecular Biology

Key Words: GST-NSD1, Pool B, Western Blot, Immunoprecipitation

Background: The NSD family is characterized by an evolutionally conserved SET domain which allows them to exert histone lysine methylation in epigenetic regulation. Alterations in NSD members are linked with developmental diseases and multiple cancers. Presently we are interested in how NSD1 functions in androgen receptor transactivation, which is critical for prostate cancer cell proliferation. Western Blot(WB) and Immunoprecipitation(IP) are both indispensable tools for the ongoing study on molecular interactions involve proteins, but their requirements for antibody are not exactly the same. An antibody competent for both techniques is badly desired. The lab PBC has already purified a guinea pig raised antibody against recombinant GST-NSD1 ----"Pool B". Now it's time for candidate evaluation.

Experiments : ① Check the over expression of GST-NSD1 in host cell(BL 21) extract by WB against Pool B (the non induced as negative control and WB against anti-GST as reference)

② IP the fusion protein from cell extract by Pool B (normal IgG as negative control), the precipitated was characterized by WB (against Pool B & against IgG) ;(GSH-agarose beads purified protein as reference)

Results & Discussion: Pool B can recognize GST-NSD1, and it exhibit high specificity in WB and IP consistently (no cross reactivity has been discovered so far), but its values in the two techniques are different. In WB, it out perform anti-GST by its high specificity with no compromise of sensitivity. While in IP, its binding with GST-NSD1 is challenged, implying a low intrinsic affinity. It need high concentration of antigen to facilitate interaction and the immune complex is quite sensitive to high salt wash buffer. Encouragingly, Pool B is able to perform IP and we are on the way to capture the optimal experimental condition.

Conclusion: Pool B is a reliable primary antibody for WB. It can serve as a detection reagent to monitor the purification of protein and do some qualitative or semi quantitative analyses. For IP, more trails are needed to get satisfactory yields, then its application spectrum will be broadened to affinity purification, analysis of protein-protein interaction and so on.

ABSTRACT

Treatment for type B aortic dissection patients

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Key Words: dissection, type B, malperfusion, endograft, medication

Background: Aortic dissection distal to the left subclavian artery (type B) is generally medically managed. Surgical intervention is required in cases of medical failure, malperfusion, rupture, and aneurysm formation. Aortic endografting is a new method for treating acute aortic dissection type B (AADB). Our aim is to identify patients eventually requiring surgery to predict those who may benefit from early aortic endografting.

Methods: We identified AADB patients from hospital admission records from 1999 to 2012. Patients with aortic branch occlusion causing malperfusion syndromes were also identified. Records were accessed to determine short- and long-term outcomes including those patients eventually treated for aneurysm repair. Regression analysis was performed to correlate factors associated with failure of medical management.

Results: Over 2000 patients were admitted for treatment acute aortic syndromes. About 400 were for proximal aortic diseases and approximately 600 were for AADB. Less than 10% required acute surgical intervention but up to 20% eventually required repair of the descending thoracic aorta due to aneurysm formation. Eight patients received an aortic endograft for rupture or malperfusion syndrome.

Conclusion: Medical management is successful in the majority of patients with AADB. Aortic endografting is an emerging technique to treat the few patients who require urgent surgical intervention. Ongoing trials are underway to determine the best candidates and outcomes.

ABSTRACT

Unidirectional Barbed Suture Versus Interrupted Vicryl Suture in Vaginal Cuff Healing After Robotic-assisted Laparoscopic Hysterectomy

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Class of 2014

Sponsored by: Vaseem Ali, MD, Department of Obstetrics, Gynecology and Reproductive Science

Key Words : V-loc, Vicryl, Vaginal cuff healing, RATH

Study Objective: To estimate whether unidirectional barbed suture has a better performance on vaginal cuff healing after robotic-assisted laparoscopic hysterectomy versus Vicryl suture.
Methods: It is a retrospective cohort study of total 93 patients who underwent robotic assisted laparoscopic hysterectomy (RALH) in our institute from July 2008 to June 2012. In 44 of the patients, the vaginal cuff was closed by an interrupted 0 Vicryl suture. In the rest 49 patients, a unidirectional barbed suture (V-loc) with a running fashion is used in cuff closure. Patients were seen 2 and 6 weeks postoperatively to evaluate the cuff healing.

Results: Age, tobacco use, hemoglobin, vaginal deliveries, menopause, steroid use, underlying health problems, concomitant procedures were found not to be significantly different between each group. There is only one cuff dehiscence in V-loc in the total 93 cases, and is found not to be significant. The mean cuff healing time is 8.5 weeks in Vicryl group and 7.7 weeks in V-loc group. In Vicryl group and V-loc group, cellulitis postoperative bleeding is all found to be not significant in statistics. However, the Vicryl is associated with more presence of granulation tissue versus V-loc (27.3% vs. 8.2%, OR=3.34, P<0.05). V-loc is also contributes to less mean surgery duration (220.2min vs. 272.8min) and blood loss (163.2 ml vs. 274.9ml).

Conclusion: In our study, V-loc is superior to Vicryl for it is associated with less presence of granulation tissue, shorter surgery duration, and less blood loss. However, there is no statistically difference in the cuff healing time, cuff dehiscence, cellulitis and postoperative bleeding between the two groups.