summer RESEARCH program 2001

student abstracts

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The University *of* Texas
Health Science Center at Houston

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PREFACE

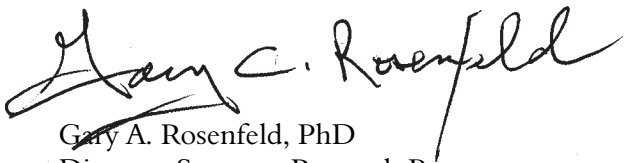
The University of Texas Health Science Center at Houston (UT-Houston) Summer Research Program provides intensive, hands-on research training for medical and undergraduate students under the direct supervision of experienced faculty researchers and teachers. These faculty members' enthusiasm for scientific discovery and commitment to teaching is vital for a successful training program.

The trainee's role in the laboratory is to participate to the fullest extent of his or her ability in researching and designing a research project, carrying out the technical aspects of experimental analyses, interpreting data, and summarizing results. These abstracts were written in the trainees' own words and convey an impressive degree of understanding of the complex projects in which they were involved.

To date, more than 1,100 students have gained research experience through the UT-Houston Summer Research Program. Past trainees have advanced to pursue research careers in the biomedical sciences, and medical students gain an appreciation of the relationship between discoveries of fundamental research and medical diagnosis and treatment.

Research training opportunities exist because of institutional support from UT-Houston schools, departments, and grants secured by the mentors, as well as support from UT-Houston's InterCon. We also acknowledge the National Institutes of Health, including the National Institute of Diabetes and Digestive and Kidney Diseases and the National Heart, Lung and Blood Institute; National Science Foundation; Texas Higher Education Coordinating Board; American Heart Association; National Aeronautics and Space Administration; and the Arthritis Foundation for their encouragement and support of our training efforts.

Science education remains a vital and integral part of our nation's interests. The UT-Houston Summer Research Program and the dedication of our faculty exemplify the institution's commitment to training and educating the future leaders of our scientific communities.



Gary A. Rosenfeld, PhD
Director, Summer Research Program
Assistant Dean for Educational Programs

ACKNOWLEDGMENTS

This publication marks the completion of the seventeenth year of The University of Texas Health Science Center at Houston's Summer Research Program. The longevity and success of the program are rooted in the overwhelming support received from the faculty, staff, and students of UT-Houston.

Indicative of this support is the funding provided by The University of Texas-Houston Medical School. Sincere appreciation is expressed to L. Maximilian Buja, MD, Dean, UT-Houston Medical School, for his support of the Summer Research Program and his commitment to research training.

In addition to the funding provided by the UT-Houston Medical School, the program is supported by the National Institutes of Health, National Research Service Award Institutional Short-Term Training Grant (DK07676) from the National Institute of Diabetes and Digestive and Kidney Diseases.

Sadly, the program lost one of its strongest supporters in 2001, Dr. Thomas F. Burks who served as Executive Vice President for Research and Academic Affairs at The University of Texas Health Science Center at Houston. Dr. Burks was a distinguished scientist who believed that attracting students to laboratory research was essential for the future of medicine. He was actively involved with the Summer Research Program each year, and will be sorely missed.

Dr. Gilbert A. Castro and Ms. Debra Samuels who, respectively, served as Director and Coordinator of the Summer Research Program, must also be recognized for their outstanding contributions. Although they will leave the program this year, collectively, they have nurtured and guided it for nearly seventeen years. In 2001, their commitment to the program was severely tested as the Texas Medical Center was one of the hardest hit areas of the historical June flood. With their strong support, students in the program were relocated to functioning laboratories to continue, and, in most instances, complete their research projects. The efforts of Dr. Castro and Ms. Samuels were deeply appreciated by the student researchers, who themselves must be congratulated for their perseverance in the face of an enormous challenge.

MEDICAL SCHOOL

EDUCATIONAL PROGRAMS

Is the non-emergent ED patient really inappropriate?

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Sponsored by: Andrew Harper, MD, Educational Programs; Todd Hamel, MD, OHSU Family Medicine
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: inappropriate, misuse, emergency department, minor injury, survey, administration

Emergency department (ED) overcrowding is a national concern directly resulting in a negative impact on the quality of care provided to patients. Contributing to this phenomenon is the presentation of excessive numbers of patients with minor problems to the ED. These patients have been labeled, "inappropriate" users of the ED, and past literature estimates from 11% to 82% of ED visits may fall into this category. Considerable research has gone into characterizing demographic groups of patients most responsible, in addition to programs that might effectively deter this practice. An extensive literature review has illuminated some concerning detours in the road to finding a solution to this problem. In defining the "inappropriate" ED patient, the majority of past studies have failed to use a negative control (i.e., a comparison group study of patients that "correctly" sought minor injury care in sources alternative to the ED). In addition, programs aimed at decreasing non-urgent patient visits to the ED have reported conflicting results. A comparative cohort study involving patients with the same chief complaint presenting to either the hospital ED or a primary care clinic was designed to identify the demographic makeup of what has been called the "inappropriate" patient. Incorporated into this project is an online survey, created to poll hospital ED administrators and primary care providers to ascertain the effectiveness of patient programs aimed at reducing ED use, their perceived risk for the patient and the opportunity cost of such care in both venues. The project should ultimately result in statistically relevant data that identifies the demographic makeup of what has traditionally been called the "inappropriate" patient. Merging this picture with the pooled opinions of patient providers should indicate the most effective way to make the inappropriate patient appropriate.

FAMILY PRACTICE AND COMMUNITY MEDICINE

Prevalence of intimate partner violence against women in a public health clinic: pre- and post-implementation of routine screening program

Erica P. Lin, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Janet Y. Groff, MD, MSPH, PhD, Family Practice and Community Medicine
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: intimate partner violence, women, abuse assessment screen

The high prevalence of violence against women has prompted an emphasized need for routine abuse assessment of all women. Nevertheless, screening is usually left to the discretion of health care providers; only a small percentage of patients presenting to primary care clinics are ever screened for intimate partner violence (IPV). The use of a standard abuse assessment protocol in patient evaluation at primary health clinics should increase detection of abuse.

The study was conducted at a public health clinic currently involved in a violence intervention project that requires women ages 18 to 44 presenting to the clinic be administered an Abuse Assessment Screen. Prevalence prior to the implementation of routine screening was determined by a retrospective chart review of 435 medical records of female patients ages 18 to 44 for indications of IPV during patient encounters within the year prior to project implementation at the clinic. Post-implementation data was taken from prevalence of IPV found during screening for program participants.

Three hundred and seventy-two patient charts have been reviewed to date, yielding a 0.54% IPV detection rate. Only 34% of the women were ever questioned about violence. In contrast, screening for project participants resulted in a 5.4% detection rate of positive IPV within the last 12 months. Preliminary results demonstrate that the incorporation of an abuse assessment protocol into starndard procedures for patient evaluation improves detection of IPV, allowing physicians more opportunities to intervene on behalf of those experiencing abuse.

Gender differences in the correlation between self-perceived physical and mental health in older adults

Kim M. Nguyen, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Linda Z. Nieman, PhD, Family Practice and Community Medicine
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: gender, older adults, mental health, physical health

Background: Only a small number of studies have been conducted regarding gender health differences among older patients. **Purpose:** This study examined gender differences in the association between self-reported physical (PCS) and mental health status (MCS) in older adults. **Hypothesis:** We believed that men would have a stronger correlation between self-perceived physical and mental health than women. **Methods:** The Short Form 36 (SF-36) questionnaire was administered in English and Spanish over a period of 8 weeks to 200 indigent patients between the ages of 55 and 64 in two Houston area clinics. The form measures PCS and MCS. The PCS and MCS scores were divided into quartiles separately for men and women. **Results:** We did not find a general association between PCS and MCS; however, correlations for patients with scores in the highest quartiles were statistically significant ($p < 0.05$). Women with PCS scores greater than the 75th percentile had a positive correlation between PCS and MCS ($p < 0.05$). However, men with PCS or MCS scores greater than the 75th percentile had significant negative correlations between PCS and MCS ($p < 0.05$). **Conclusion:** The positive correlation between PCS and MCS demonstrated by women supports previous research suggesting that there is a causal relationship between PCS and MCS. However, the negative correlation between PCS and MCS demonstrated by male patients suggests that physicians cannot ignore the treatment of mental health in men who present with good perceptions of their physical functioning.

Student barriers to preventive practices during summer preclinical preceptorship

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Sponsored by: Linda Z. Nieman, PhD, Family Practice and Community Medicine
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: medical students, preceptorship, diabetes, barriers

Diabetic foot exams have been shown to decrease patient morbidity and foot amputation. During the summer Texas Statewide Family Practice Preceptorship Program (TSFPPP) that emphasized preventive practices to improve diabetic care, medical students completed 55 of 600 possible diabetic foot examinations. The objective of this cross sectional study was to identify the main barriers to fulfilling suggested preventive medicine activities that medical students faced during their preceptorship experience. Fifty-two medical students participating in 60 TSFPPP four-week preceptorship programs (eight students participated in two preceptorships) were interviewed by phone and/or received a formal comparable questionnaire by email at the end of their preceptorship. Answers were assessed and categorized utilizing content analysis techniques and QSR NUD*IST. Three themes materialized as barriers to completing this preventive practice: (1) physician time constraints, (2) variety in practice types, and (3) lack of student/preceptor interest and self-efficacy in performing the diabetic foot exam. Specific recommendations are necessary to encourage students to overcome barriers to achieving self-efficacy and beginning to assist with preventive practices in the preceptors' offices.

INTEGRATIVE BIOLOGY AND PHARMACOLOGY

Short-term and long-term effects of heat stress

Andrea L. Pingitore, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Edgar T. Walters, PhD, Integrative Biology and Pharmacology
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: heat stress, heat shock, input resistance, threshold

Many cells show short-term and long-term abilities to adapt to stress, including heat. Heat stress induces many cell reactions including the synthesis of heat shock proteins. Intracellular studies of identified *Aplysia* neurons may provide clues about mechanisms underlying cellular stress responses. To see if heat shock affects electrophysiological properties, the intact animal was heated to 35°C for 20–40 minutes. Intracellular recordings were made in ganglia after application of one or two heat shocks. The ganglia were tested 24 hours after heating the body or one hour after heat in the isolated ganglion a second time after the initial heating 24 hours earlier. Mean input resistance of ganglia heated once was 11.4 MΩ, the ganglion heated twice was 9.6 MΩ, and the control, 14.7 MΩ. Mean threshold current necessary to evoke an action potential in controls was 0.52 nA, the once heated ganglion was 0.67 nA, and the twice-heated ganglion was 0.74 nA. Thus, sensory neurons responded to heat stress by raising threshold of action potential, or input resistance. It will be interesting to see whether these changes involve mechanisms that are also triggered by other forms of stress, such as axotomy.

INTERNAL MEDICINE

Downregulation of glucose transporter 4 gene expression in human diabetic cardiomyopathy is masked by ischemia

Tonya C. Cockrill, The University of Texas Medical School at Houston, Class of 2004

Third Place 2001 Frank Webber Prize for Student Research
Sponsored by: Heinrich Taegtmeyer, MD, DPhil, Internal Medicine—Cardiology
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35KD07676
Keywords: heart failure, diabetes, clinical database, gene expression

Background: Heart failure in patients with diabetes (diabetic cardiomyopathy) is associated with a more severe prognosis than heart failure in patients without diabetes. This study characterizes diabetic and non-diabetic patients at the clinical level (using parameters of heart function) and at the molecular level (measuring gene expression of key regulators of energy substrate metabolism). **Methods:** Clinical data of 22 patients with end stage cardiomyopathy, 5 with insulin dependent diabetes mellitus, 8 with non-insulin dependent diabetes mellitus, and 9 without diabetes, were collected from medical records at the Texas Heart Institute and entered into a database. Myocardial tissue (LV apex) was obtained during left ventricular assisted device implantation. RNA was isolated, and transcript levels of metabolic genes were measured using real time quantitative RT-PCR. **Results:** Demographic data (gender, age, and ethnicity) did not differ between groups. All patients showed impaired left ventricular function, including decreased cardiac output, decreased cardiac index, decreased ejection fraction, and increased left ventricular diastolic dimension. Transcript levels of glucose transporters (GLUT 1 and GLUT 4), long chain fatty acyl-CoA transport (mCPT-1), and long chain fatty acid oxidation (MCAD) were not different between groups. However, subset analysis of non-diabetic and diabetic patients with non-ischemic heart disease revealed that GLUT 4 gene expression is significantly downregulated in diabetic patients. **Conclusions:** Transcript levels of GLUT 4 are downregulated only in diabetic patients free of coronary heart disease, suggesting that ischemia masks diabetes induced alterations in metabolic genes. The clinical significance of this downregulation of GLUT 4 expression is being evaluated.

Does antiangiogenic treatment of human tumors with endostatin change blood flow in non-tumor muscle tissue?

Keagan H. Lee, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Nizar A. Mullani, Internal Medicine
Supported by National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: endostatin, angiogenesis, tumors, positron emission tomography (PET)

In preclinical studies, endostatin has been demonstrated to cause tumor regression by inhibiting angiogenesis. A recent Phase I dose-escalating study of endostatin in cancer patients showed a dose-dependent decrease in blood flow in tumors. Our goal is to determine if there is any change in blood flow to non-tumor tissue following treatment with endostatin.

Blood flow images measured by PET in a phase I endostatin study were analyzed by drawing a region of interest over muscle tissue at baseline and 28 days after endostatin treatment in 18 patients. Data were analyzed using the paired t-test for means for all the patients, and then subdivided into a low dose group of below 120 mg/m^2 and greater than 120mg/m^2 of endostatin.

The mean muscle blood flow at baseline for all patients was 4.1ml/min/100gm of tissue and increased to 7.2ml/min/100gm of tissue after endostatin, with a p value of 0.07. The mean blood flow for the low dose group went from 4.3ml/min/100 gm of tissue to 3.9ml/min/100 gm after endostatin, and for the high dose group increased from 4.0ml/min/100gm of tissue to 9.2ml/min/100gm of tissue with a p value of 0.06.

The slight blood flow increase in muscle tissue of patients receiving high doses of endostatin was not found to be statistically significant, yet additional data from a larger number of patients, and involving other non-tumor tissue, are necessary to fully answer this question.

Evaluation of the efficacy of the fusion inhibitor T-20

Suong M. Tran, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Ben J. Barnett, MD, Internal Medicine—Infectious Diseases
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: HIV/AIDS, fusion inhibitor, T-20

This study evaluated the efficacy of the fusion inhibitor T-20 in highly antiretroviral experienced HIV infected patients. 11 patients were enrolled, but 2 were lost to follow-up. Patients were randomly assigned to receive T-20 or to be in a control group, which did not receive T-20. All patients were on an optimal background antiretroviral regimen. Of the remaining patients, 3 were assigned to control, 6 to T-20. T-20 was given at a dose of 90mg SQ BID. Patients are to be followed biweekly for 6 weeks. Results of the 6-week data showed the mean VL_{log} at the outset to be 4.93 for the T-20 patients and 5.02 for the control patients. The mean decrease in VL_{log} at 6 weeks was 2.52 for the T-20 patients and 1.37 for the control patients. None of the patients achieved log VL < 1.69 during the six-week study period. No serious adverse effects to T-20 were noted. As expected, all patients on T-20 experienced SQ nodules. Thus, T-20 appears to be safe when given in addition to optimal background antiretroviral regimens. It appears to be successful in the suppression of viral replication. Further follow up is necessary to discover the long-term efficacy of this new fusion inhibitor.

MEDICAL GENETICS

Thoracic aortic aneurysms and dissections; characterization of the clinical phenotype of the families linked to 5q, 11q, and not linked to either locus

Danielle K. Lemuth, The University of Texas Medical School at Houston, Class of 2004

First Place 2001 Frank Webber Prize for Student Research
Sponsored by: Dianna M. Milewicz, MD, PhD, Medical Genetics
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: thoracic aortic aneurysms, aortic dissections

Familial non-syndromic thoracic aortic aneurysms/dissections are defined by patients that have one or more first-degree relatives with aortic aneurysms/dissections, but who do not meet the current diagnostic criteria for the Marfan syndrome. Preliminary studies of clinical phenotypes have shown correlations between TAA families linked to 5q and the presence of a bicuspid aortic valve and/or the occurrence of cerebral aneurysms (although we do not yet have the DNA samples to confirm 5q inheritance with the cerebral aneurysms). Families with a history of TAA are currently being identified and recruited for this study through surgical records and referrals. All of the families collected are being used to test the hypothesis that there are differences in the aortic diseases or other phenotypic manifestations in families in which there is linkage of the phenotype associated or not associated with specific loci. For these studies, a Multipoint LOD score is determining linkage to the 5q or 11q loci. Univariate analysis involving chi-square analysis and ANOVA are being used to determine means and frequencies within and between these groups using a commercial software program (SAS, version 8.0). The Bonferroni test is being used to determine differences between means in multiple groups. While this study has not yet reached a point of conclusion, if it does confirm the clinical phenotype associated with each loci, this information can be used in the future in genetic counseling of TAAs families.Neurobiology and Anatomy

NEUROBIOLOGY AND ANATOMY

Visual Exploration of Cranial and Cervical Neurovascular and Skeletomuscular Structures

Timothy Davenport Spires, Jr., The University of Texas Medical School at Houston, Class of 2004

Sponsored by:Len Cleary, PhD, Neurobiology and Anatomy
Supported by: The University of Texas-Houston Summer Research Program, National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: neurovascular, skeletomuscular, anatomic illustrations, cranial nerves, infratemporal fossa

The study of the head and neck has been a challenge to those entering the field of medicine since time immemorial. These two regions of the body are composed of a complex architecture of neurovascular and skeletomuscular structures that are woven together in an almost seamless manner. The goal of this project is to generate novel anatomical illustrations that elucidate the key elements of these structures while maintaining the spatial relationships of the cranial and cervical regions. The resulting anatomical illustrations, which combine hand rendering with computer manipulation, seek to clearly portray the underlying anatomy of the head and neck. Each individual drawing focuses on a different aspect of the anatomy. The cranial nerves are inherently difficult structures to visualize and so were a priority of this project. Another problem area is the infratemporal fossa, which contains an assortment of nerves, vessels, and muscles discretely lodged under the bones of the face. The structures of the ear and orbit were also rendered. The ultimate goal of this project is to supplement the available materials of the Gross Anatomy curriculum and ease the difficulty of learning the anatomy of the head and neck.

NEUROLOGY

Analysis of temporal and spatial activation of transcription factor NF-kB following experimental ischemic stroke using transgenic mice – implication to neuronal death

Richard Lebow, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Jaroslaw Aronowski, PhD, Neurology
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: NF-κB, ischemic stroke, transcription factors

Inflammation occurs in response to ischemic stroke and has been shown to produce deleterious effects. The ubiquitous transcription factor nuclear factor-kB (NF-κB) regulates expression of many pivotal mediators of the inflammatory response in CNS injury. The relationship between NF-κB activation and cellular loss was established by comparing the cells in which NF-κB activation occurred to where cell death occurred. These laboratory studies help develop an understanding of the pathologic processes of ischemic stroke, which may provide the framework for developing new therapies that when combined with reperfusion therapy could improve the outcome after stroke in humans. NF-κB reporter transgenic mice, which produce ß-galactosidase upon NF-κB activation, were utilized in order to localize NF-κB activation at both the anatomical and cellular level. Ischemic stroke was produced by unilateral tandem occlusion of the middle cerebral and common carotid arteries. Several groups of animals were given ischemia for specified duration, with some having reperfusion after the stroke. Analysis of the staining for cell type (e.g., neuron, astroglia, endothelium), cellular death (TUNEL), and for ß-galactosidase made it possible to determine when and what cells displayed NF-κB activation and cell death. While this research is still in progress, it has been hypothesized that the acute phase response to ischemic stroke is in part a dynamic inflammatory response coordinated by the transcription factor NF-κB. In addition, early and prolonged NF-κB activation leads to neuronal death; therefore, the spatial distribution of early NF-κB activation will positively correlate with a distribution of neuronal death.

OBSTETRICS AND GYNECOLOGY

Cyclin B1 as a surrogate endpoint biomarker in cervical intraepithelial lesions in a chemopreventive prospective

David D. Hamilton, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Michele G. Curtis, MD; Anne-Therese Vlastos, MD, Gynecology and Obstetrics
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Key words: cyclin B1, cervix, biomarkers

Cyclin B1 is a possible biomarker whose status could prove to be valuable to cervical chemoprevention. The aim of this study was to determine the feasibility of cyclin B1 as SEB in cervical chemoprevention trials.

In order to discover effective chemopreventive agents, we must find suitable biomarkers that give accurate reflections of the status of the tissue or the level of dysplagia. Cervical lesions may be monitored to study cancer progression. Cervical lesions are easily accessible for observation by colposcopy and for cytological sampling by biopsy or Pap smear. Detectable changes indicating progression of disease are observable by both cervical biopsy and Pap smear. Surrogate endpoint biomarkers (SEB) are a practical necessity to monitor prospective chemopreventive agent efficacy, and allow costly and lengthy clinical trials to be conducted within a realistic time frame. Good SEBs are differentially expressed in normal and high risk tissues, appear at well defined stages of carcinogenesis, should be easily measurable with limited variability, and modulation of SEBs by chemopreventive agents should indicate a decrease in cancer incidence rate (1). Here we hypothesize that cyclin B1 is possibly an appropriate SEB for cervical chemoprevention trials and we have attempted to determine its expression in tissue of normal, CIN I, CIN II,CIN III, and tumor cervical sections by IHC and determine if there is a correlation with prognosis.

Cyclins and CDKs are upregulated in cervical dysplagia (2). Altered regulation of the cell cycle is a hallmark of human cancers (3). Overexpression of cyclin B1 has been recently demonstrated in colorectal, prostate, breast, esophagus, and head and neck cancers as well as Hodgkin and MALT lymphomas (4-10). Its prognostic value has been suggested in patients with SCC of the esophagus (8). Now, Sorria et al (11) have demonstrated that cyclin B1 is overexpressed in a significant fraction of lung

carcinomas, especially NSCLCs, and that high cyclin B1 expression is a significant unfavorable prognostic factor in patients with stage I NSCLC. These results were accompanied by the absence of prognostic value for Ki-67 , suggesting that high cyclin B1 expression in NSCLCs is not a mere consequence of cell proliferation, but rather an indicator of aberrant cell cycle progression at the G2/M transition in cancer cells (11). High level of cyclin B1 is likely to cause a longer mitosis and increase mitotic defects and chromosome aberrations (12). We chose to examine cyclin B1 because of its aberrant expression has been shown in other epithelial carcinomas such as lung, oral, and esophageal.

Materials and Methods: Immuno-histochemical Staining for Cyclin B1, Cyclin E and Ki-67 Protein. Paraffin-embedded, 4-mmm-thick tissue sections from normal tissue, CIN I, II, III, and tumor were stained for the cyclin B1 protein using a primary mouse monoclonal antibody (NCL-Cyclin B1, Novocastra, Newcastle, UK) and for Ki-67 by using a primary rabbit polyclonal antibody (Dako, Carpinteria, CA). Slides were baked at 55°°C overnight and then deparaffinized through a series of xylene baths. Rehydration was performed through graded alcohols. To retrieve the antigenicity, tissue sections were then treated with microwaves in 10 mM of citrate buffer (pH 6.0) for 5 min, three times for cyclin B1, and six times for Ki-67. The sections were then immersed in methanol containing 0.3% hydrogen peroxidase for 10 min to block the endogenous peroxidase activity and were incubated in 5% blocking serum to reduce nonspecific binding. Sections were incubated for one hour at room temperature°° with primary anti-cyclin B1, or anti-Ki-67 antibody at dilutions of 1:50, and 1:100 respectively. The sections were then processed using standard avidin-biotin immunohistochemistry according to the manufacturer's recommendations (Vector Laboratories, Burlingame, CA). DAB was used as a chromogen, and hematoxylin was used for counterstaining. Tissue sections of normal lymph node were used as positive staining controls for cyclin B1. Sections were also stained with the primary antibody omitted to confirm staining specificity.

Results: Preliminary analysis indicate a trend from normal tissue to CIN III cervical tissue showing an increase in cyclin B1 immunohistochemical staining, and indicating an increase in its' expression. This increase does not seem to correlate with Ki67, indicating an independent proliferation of tumor and that high cyclin B1 expression is not consequence of cell proliferation, but rather an indicator of aberrant cell cycle progression at the G2/M transition in cancer cells. Up till now the slides have been scored by a single observer and we are in the process of scoring them a second time by a different scorer. Should these results be confirmed, Cyclin B1 appears to be a relevant biomarker in chemopreventive trials because of its' differential expression in cervix.

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Analysis of gender and cardiovascular disease in the Cochrane Reviews

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Sponsored by: Shahla Nader, MD, Obstetrics and Gynecology; Cynthia L. Phelps, PhD, Health Informatics; Barbara M. Sanborn, PhD, Biochemistry and Molecular Biology
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: cardiovascular disease, Cochrane, gender, women

Women have different risk factors for cardiovascular disease than men, commonly present with a unique set of symptoms, and treatment outcomes may vary for women. Cardiovascular disease is the number one killer of females in this country, yet the health care of women is often based on evidence from clinical trials performed predominantly on men. The Cochrane Library, published quarterly online, is the definitive source for evidence-based medicine: it maintains a large database of controlled clinical trials and publishes meta-analyses addressing various topics called "Systematic Reviews." Thirty Cochrane reviews (published by the Cochrane Heart Group, Hypertension Group, and Peripheral Vascular Diseases Groups) and their references were carefully examined for inclusion of gender-specific information on cardiovascular disease. Specifically, the 30 meta-analyses collectively pooled data from 299 clinical trials, and the original publications for those trials were consulted to see how many female subjects were included and if a data analysis was performed by sex. In the trials we reviewed, women made up approximately 29% of the population, and 30% of the clinical trials including women published a data analysis by gender. About one-fifth of the studies that did a gender-based analysis found that cardiovascular outcomes varied significantly ($p < 0.05$) by sex. We conclude that all clinical trials including women need to analyze data by sex and that the Cochrane Systematic Reviews should include this gender-specific information so that this source of evidence-based medicine will apply as much to women as to men.

OPHTHALMOLOGY AND VISUAL SCIENCE

Ocular effects of celecoxib after oral administration

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Sponsored by: Robert Feldman, MD, Ophthalmology and Visual Science
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: celecoxib, NSAIDs, radioimmunoassay

In the eye, current non-steroidal anti-inflammatory drugs (NSAIDs) are available for topical use in the form of eye drops. They are used in ocular procedures for their anti-inflammatory and antimiotic effects. NSAIDs block the production of prostaglandins by inhibiting both isoforms of the enzyme cyclo-oxygenase, COX-1 and COX-2. However, because NSAIDs block the COX-1 enzyme, they can cause ulcers, along with other adverse effects such as conjunctival and scleral melting. Celecoxib, a specific COX-2 inhibitor, has been shown in a controlled trial to have an eight-fold lower incidence of upper gastrointestinal ulcer complications than non-specific NSAIDs (Goldstein, Silverstein, Agarwal, 2000). Celecoxib, if shown to have adequate ocular penetration after oral administration, may provide another drug for use in ocular procedures without the toxicity of NSAIDs.

In the current, ongoing clinical study, 30 patients are being studied to determine if Celecoxib penetrates ocular tissue. Group 1 is made up of five patients with glaucoma who are scheduled for trabeculectomy. Group 2 is comprised of five patients with retinal disease who will undergo vitrectomy. Group 3 is comprised of five patients with cataracts who are scheduled for cataract surgery. Five age-matched controls are also enrolled for each group. The study subjects are being placed on celecoxib 100mg twice daily for five days preoperatively, while the control group is receiving a placebo. All subjects are having 0.1mls of aqueous humor collected during the procedure, which is being tested by radioimmunoassay to determine levels of Prostaglandin E_2 and 6-Keto-prostaglandin F_1?. The prostaglandin levels are expected to fall in subject eyes compared to control eyes if celecoxib has penetrated into the eye.

ORTHOPAEDIC SURGERY

Thermal Capsular Shrinkage in Glenohumeral Instability

Robert K. Fullick, The University of Texas Medical School at Houston, 2004

Sponsored by: Catherine Ambrose, PhD, Orthopaedic Surgery
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Key words: thermal capsular shrinkage, radiofrequency energy, glenohumeral instability, arthroscopic surgery, biomechanics

The purpose of this project is to evaluate the effectiveness of thermal capsular shrinkage in the management of glenohumeral instability. Specifically, we plan to further quantify the reduction in translation that can be accomplished using a radiofrequency probe to heat different portions of the capsuloligamentous structure of the shoulder. This is a biomechanical cadaveric study testing the changes in glenohumeral translation in the anterior and posterior direction after the treatment of the posteroinferior shoulder capsule with a radiofrequency probe. Fresh frozen cadaveric shoulders will be thawed and stripped of all soft tissues except the capsule and ligaments. Each specimen will be mounted in a jig connected to an x,y table and motion sensor capable of measuring translation in the anterior and posterior direction. Anterior and posterior directed loads will be applied through pulleys and translation recorded before and after heat shrinkage. Translation will first be measured in both the anterior and posterior direction for the untreated specimens. Various regions of the capsule will be heated under direct visualization by the radiofrequency probe. After treatment with the RF probe, the cadaveric shoulders will then be replaced exactly as before. Once again translation will be measured. Each time the specimens will be subjected to a 15-N and a 20-N load in both directions. A paired t-test will be used to compare joint translation before and after treatment, as well as joint translation with the 15-N and 20-N forces. After mechanical testing is complete, tissue samples from both the treated and untreated regions will be taken for histological evaluation. Analysis of these samples will focus on the effects of thermal application on the collagen structure of the tissue. Results and conclusions will be drawn upon the subsequent completion of biomechanical testing in the near future.

Outcome of hip fractures in men

Karen N. Perser, The University of Texas Medical School at Houston, 2004

Fourth Place 2001 Frank Webber Prize for Student Research
Sponsored by: Catherine Ambrose, PhD, Orthopaedic Surgery; Gary Kiebzak, PhD, Center for Orthopaedic Research and Education, SLEH
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: men, osteoporosis, hip fracture, antiresorptive medication

Osteoporotic hip fractures result in significant morbidity and mortality. Historically, research has focused on aspects of osteoporosis in women. Several antiresorptive therapies are now available to protect women against bone mineral density loss. It is our belief that the prevalence of osteoporosis in men is under appreciated. The purpose of this study was to determine if men are being treated for osteoporosis after hip fractures. We conducted a retrospective chart review of patients who presented with hip fractures to St. Luke's Episcopal Hospital between 1996 and 2000, followed by mail-out survey to acquire information about the outcomes of hip fractures in men ($n = 113$) and women ($n = 258$). Completed surveys were returned by 150 patients. Twelve months postfracture, the mortality rate was greater in men (31%) than women (17%), ($p < 0.004$, Fishers exact test). After 1 to 5 years, 56.6% of men and 41% of women were deceased ($p < 0.007$, men vs. women). Of women with a hip fracture, 23.3% were discharged from the hospital on some type of antiresorptive medication, while only 3.5% of men with a hip fracture received antiresorptive medication ($p < 0.001$). Based on survey information, 48.1% of women and 9.5% of men later began antiresorptive treatment within 1 to 5 years after fracture ($p < 0.001$). Based on these results, we conclude that men are not methodically treated for osteoporosis. Future research efforts should be designed to modify the clinical pathway for hip fracture to ensure more aggressive medical treatment for osteoporosis.

Comparison of single pole vs. triple pole polyethylene patellar implant fracture resistance: A cadaveric biomechanical evaluation

Jeevan Ramakrishnan, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Terry Clyburn, MD, Orthopaedic Surgery
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676

Approximately 220,000 total knee replacements are done in the U.S. per year, and fracture of the patella is a known complication of this procedure. This complication may lead to significant functional disability and may even require further revision of the implant. The purpose of this study is to determine whether the model of patellar implant affects performance or significantly alters fracture resistance. The results could then be used to reduce the incidence of this complication and significantly improve the patients' lifestyles. Two designs of the patellar implant will be compared – a single pole design and a triple pole design. Using matched pairs of patellae, each pair of patellae will receive one single pole and one triple pole design, which will be implanted randomly on either the right or left knee. The revised patellae will be placed in contact with a femoral total knee component, and fracture will be induced by dropping standard weights from a set height onto the implant. Patellar fracture will be assessed visually after each blow, and the force required to cause fracture will be determined. The data will then be analyzed statistically using a paired t-test to formulate a conclusion. We hypothesize that the fracture rate is more prevalent in the single pole design due to the greater resection of bone. Therefore, the triple pole design should prove to be more stable, and the results will encourage the use of this design in total knee replacements.

Quantitative analysis of the osteoarthritic changes of the first metatarsophalangeal and subtalar joints

Thomas L. Tanous, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Thomas O. Clanton, MD, Orthopedic Surgery; Lawrence M. Ross MD, PhD, Neurobiology and Anatomy
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: articular cartilage, 1st metatarsophalangeal joint, calcaneofibular ligament, subtalar joint, osteoarthritic degeneration

One of the first and most common sites of osteoarthritic changes in the body is the first metatarsophalangeal joint. Much research in the study of osteoarthritic changes of the foot has centered on the sesamoids lying under the first metatarsal head. It is our intent to discover the correlation if any exists between osteoarthritic changes of the first metatarsophalangeal and disease of the sesamoids. It has also been hypothesized that ankle joint instability, particularly of the subtalar joint may also play a role in the development of osteoarthritis. The calcaneofibular ligament contributes to the stability of the subtalar joint and may have some role in the likelihood of the development of osteoarthritic changes. It is our belief that the ligament orientation and angle of insertion may play a large part in the development of ankle instability. Eighteen pairs of preserved cadaver ankles have been procured for this study. The calcaneofibular ligaments of the specimens were exposed, documented for anomalies, and ankle of insertion measured. The subtalar joint was subsequently exposed and rated for osteoarthritic changes using the Outerbridge classification system. The first metatarsophalangeal joint was similarly exposed and classified. Photographs are taken of both articular surfaces and will be used to create a composite image showing common locations of articular degeneration along the joint surface. The completion of this project will be to complete the dissections and using the data collected to make conclusions on contributing factors to the development of osteoarthritic changes seen in the foot.

Intramedullary Hip Screw Fixation at Intertrochanteric Hip Fractures

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Sponsored by: Kevin J. Coupe, MD, Orthopaedic Surgery
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: orthopaedics, hip fractures, fixation, hip screw

Purpose: Hip fractures are often a devastating injury in all age groups. Closed intramedullary nailing of hip fractures is attractive for its potential to possibly reduce the morbidity associated with open reduction and internal fixation. Consequently, the patient may experience reduced perioperative complications with a potentially faster rehabilitation. Results of the IMHS are not widely reported in the literature. The purpose of this study is to evaluate the results of intramedullary hip screw fixation in the treatment of intertrochanteric fractures.

Material and methods: The medical charts and radiographs of the patients with isolated fractures of the intertrochanteric region treated at this institution from May 1996 to April 2001 were retrospectively reviewed.

Results: This study is still ongoing. Preliminary data from 16 female and 9 male patients with a mean follow up of 6 months are given here. The average operation time for IMHS fixation was 62 ± 22 minutes with an average estimated intraoperative blood loss of 135 ± 75 ml. Of the 25 patients, 13 required blood transfusions averaging 1.2 ± 1.3 units of packed red blood cells. Current literature states an average operative time of 71 ± 28.9 minutes with an average intraoperative blood loss to be 144 ± 120.5 ml for IMHS fixation. Literature indicates average number of units of packed red blood cells transfused is 0.9 ± 0.96. In out study, the average length of hospital stay was 8 days and the average post-operative day of weight bearing was 3 days. Complications with IMHS fixation included one patient with nonunion, cut-out of the lag screw, and implant breakage, and two other patients with only cut-out. Two of these patients were treated with hemiarthroplasty. One patient had pulling-out of the compression hip-screw. Four patients died of unrelated causes during the follow-up period.

PATHOLOGY AND LABORATORY MEDICINE

Subcellular markers in ventricular myocytes as indicators of heart failure and recovery

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Sponsored by: Roger J. Bick, PhD, MIBiol, Pathology and Laboratory Medicine
Supported by National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: cardiac disease, fluorescence, microscopy, myocytes

A collaborative study with Texas Heart Institute has shown that mechanical unloading of failing hearts results in many dramatic improvements of the damaged tissue. This research is an initial attempt to decipher some of the cellular and subcellular changes that are compromised and then repaired. Subcellular makers relevant to both cardiac damage and cardiac repair were investigated, by fluorescence deconvolution microscopy, hopefully so that clinical interventions can be designed to delay cell destruction and speed recovery.

Previous work has shown that calcium-handling problems are due to membrane leakiness and that the calcium pump itself is intact and can perform well enough to maintain calcium homeostasis provided 'outside' influences are controlled. Using human heart tissue taken at the times of implant and explant of a ventricular assist device (LVAD), experiments were preformed with fresh and frozen specimens, in order to image a number of subcellular components and compounds that have been implicated to play a major role in heart failure. The interest in cytokines, iNOS and cell death markers (apoptosis v necrosis) in heart failure is a) because of previously reported work detailing their effects on calcium transients and nitric oxide and b) because of the increased levels of serum cytokines after and during cardiac ischemia.

Sensitivity of VisE antigenic variation protein versus immunodominant conserved region named IR_6 in diagnosing Lyme disease

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Sponsored by: Steven J. Norris, PhD, Pathology and Laboratory Medicine
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: *Borrelia burgdorferi*, VlsE, IR_6

Lyme disease is an arthropod-borne infectious disease caused by *Borrelia burgdorferi*. *Borrelia burgdorferi* has previously been shown to contain a variable surface antigen called VlsE. Previous enzyme linked immunosorbent assay (ELISA) results showed that VlsE may offer greater specificity than ELISAs using whole *B. burgdorferi* as the antigen. In this study ELISAs were used to determine the sensitivity of a full-length VlsE compared with that of a 26-mer synthetic peptide (C_6) representing IR_6 an immunodominant conserved region found in VlsE. Each test was standardized for optimal serum and antigen concentration. The specificities of the two assays with healthy blood donor sera were comparable. For a total of 54 Lyme disease patient sera examined, the VlsE ELISA yielded sensitivities of 75.9% compared with 90.7% for the C_6 ELISA. This difference in sensitivity may be due to loss of antibody following treatment. For a total of 36 untreated Lyme disease patient sera examined, the VlsE ELISA yielded sensitivities of 91.6% compared with 94.4% for the C_6ELISA. The higher sensitivity of the C_6 ELISA may be due to the lower serum dilution (1:200) used in the C_6 assay compared to the VlsE assay (1:800). In addition, higher concentrations of antigen were used in the (5.0mg/ml) C_6 ELISA compared with that of the (0.5mg/ml) VlsE ELISA. In summary, VlsE-His and C_6 ELISAs yielded similar sensitivities in untreated patients, but were differentially affected by treatment.

PEDIATRICS AND MEDICAL GENETICS

Methylene tetrahydrofolate reductase genetic polymorphisms and spina bifida

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Sponsored by: Hope Northrup, MD, Pediatrics and Medical Genetics
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: MTHFR, genetics, spina bifida, sequencing

The gene methylene tetrahydrofolate reductase (MTHFR) encodes for an enzyme that contributes a role in circulation and amino acid metabolism. Since the discovery of MTHFR, polymorphisms of the gene have been identified and linked to homocystinuria, preeclampsia, diabetes mellitus, methylene tetrahydrofolate reductase deficiency, and neural tube defects. Spina bifida (SB), a neural tube defect, is one of the most common birth defects in the United States (1 in 2000). Within the Hispanic population, SB incidence is 2.5 times greater. Although peri conceptional folate supplementation reduces SB up to 70%, the mechanism of action is unknown. MTHFR polymorphisms such as C677T correspond to SB phenotypes and are designated as risk factors. To identify additional polymorphisms within the gene, primers were designed for the 11 exons of MTHFR. In a cohort of 350 samples separated by Hispanic and non-Hispanic populations, the coding regions of the MTHFR gene were amplified using these primers. Sequencing of exons 1–4 was completed in the entire study population. In exon 1, a silent change (P to P) was detected in amino acid 39. The frequency of this polymorphism in our sample set is 0.8995. No changes were detected in exons 2 or 3. In exon 4, the C677T change was confirmed with frequencies of 0.575 and 0.455 in the non-Hispanic and Hispanic populations, respectively. Exons 5-11 have been amplified and will be sequenced over the next few months.

Hormonal regulation of PLAC1 gene expression

Tiffany A. Lunt, The University of Texas Medical School at Houston, Class of 2004

Second Place 2001 Frank Webber Prize for Student Research
Sponsored by: Michael E. Fant, MD, PhD, Pediatrics
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: PLAC1, placenta, KGF, forskolin

PLAC1, an X-linked gene, is expressed only by trophoblast cells in the placenta and has been implicated in placental growth and development. A human trophoblastic cell line, the BeWo choriocarcinoma cell line, displays many of the characteristics of normal trophoblastic cells. BeWo cells can be induced to differentiate by undergoing fusion and forming syncytia in the presence of cAMP activators, analogous to the normal trophoblast. Preliminary studies have demonstrated that PLAC1 is expressed by the BeWo cell. We therefore used the BeWo cell as a model system to study the regulation of PLAC1 expression. BeWo cells were grown in 100mm culture dishes in DMEM supplemented with 10% fetal calf serum. Once the cells were approximately 90% confluent, they were placed in serum-free keratinocyte basal medium (KBM) containing 0.2% BSA for 16–20 hours. They were then treated with various agents known to regulate trophoblast growth for 48 hours. Forskolin was selected as an activator of cAMP, and keratinocyte growth factor (KGF) was examined because of its ability to regulate differentiated trophoblast function. Cell treatments consisted of KGF (50ng/ml), forskolin (100uM), or KGF and forskolin together, each in the presence or absence of estradiol (10nM). Total RNA was then isolated and tested for PLAC1 expression by Northern analysis. Compared to controls, BeWo cells treated with KGF showed markedly (2–4 fold) increased expression of PLAC1. By contrast, cells treated with forskolin exhibited a decrease in PLAC1 expression compared to control cells, and attenuated KGF-stimulated expression. Studies examining the effect of estradiol on PLAC1 gene regulation have been completed but final analysis is still in process. The results thus far indicate that PLAC1 gene expression is regulated by factors that are known to regulate trophoblast growth and function and is consistent with PLAC1 playing an important role in the growth and development of the placenta.

PSYCHIATRY AND BEHAVIORAL SCIENCES

Psychiatric patient self evaluations and care giver evaluations as predictors for hospital readmission

Amy Fowler, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Patricia Averill, PhD, Psychiatry and Behavioral Sciences
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T25DK07676
Keywords: program evaluation, psychiatric hospitalization, readmission

With the induction of managed care, psychiatric hospitals are now, more than ever, obligated to evaluate inpatient programs to ensure quality and effectiveness of care. Readmission to the hospital after initial hospitalization is obviously something all inpatient programs strive to prevent. The goal of this project is to determine predictive factors present at the time of admission and discharge, which might be recognized and acted upon to decrease the likelihood of the patient returning to the hospital. At admission and discharge, patients were evaluated by physicians, nurses, and by themselves using the brief psychiatric rating scale (BPRS), the affective disorder rating scale, the brief symptom inventory, and the internal state scale. Scores from bipolar, schizophrenic, and major depression disorder patients on these evaluations were separated by diagnosis and number of readmissions within one year, and analyzed by the statistical analysis software, SPSS. Analyses showed that several self-evaluation categories were significant predictors, including phobic behavior, anxiety, depression, somatic complaints, psychoticism, as well as a global symptom score. Among bipolar and schizophrenic patients, self-evaluation scores at discharge proved to be highly predictive of rehospitalization, while certain items in the resistance subset of the BPRS physician evaluations showed significance also. This information should help identify patients at risk for readmission so that attention may be directed towards prevention.

RADIOLOGY

The use of pneumatic injury devices in the study of spinal cord trauma

Shouieb Tambra, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Ponnada Narayana, PhD, Radiology
Supported by: The National Institute of Diabetes and Digestive and Kidney Diseases, 25DK07676
Keywords: primary injury, secondary injury

The purpose of this study is to produce a consistent and quantifiable rat model of human spinal cord injury and use this model to follow the progression of injury. One key factor in being able to produce legitimate data in a study of this type is the ability to inflict consistent primary injuries to the dorsal motor regions of the spinal cord. The primary injury can be inflicted in numerous ways to the exposed spinal cord such as by electrical means, compression, or trauma. Electrical lesioning of the spinal cord works well, but has its limitations since the secondary injury which is caused by macrophage activity, cellular processes, and inflammation may vary considerably from typical injuries. Compression injury techniques use balloons to compress the spinal cord over the course of several minutes, producing a loss of function similar to that caused by tumor growth. The trauma method of injury that is used in this study was selected because it most closely represented the type of injury inflicted in the real world situations like automobile accidents, gunshot wounds, etc. The device is electronically controlled and pneumatically driven; so, it is possible to configure the injury apparatus so that the time and speed of the injury inflicted can be varied. With this device it is possible to vary the severity of the injury in a controlled way. This device also has the ability to digitally record data about the injury and dump it to any PC equipped with a data acquisition card and the appropriate software, an option that is not possible with other injury techniques. In conclusion the electronic pneumatic injury device should prove to be an invaluable tool for spinal cord injury research.

SURGERY

Endoscopic ultrasound in the evaluation of pancreatic cancer resectability

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Sponsored by: Craig P. Fischer, MD, MPH, Surgery
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: pancreatic cancer, resectability, endoscopic ultrasound

Tumor resectability in pancreatic cancer patients is dependant upon invasion of either the superior mesenteric artery (SMA) or portal vein. Using the Lawyer grading system, staging of tumor invasion has been made possible using various investigative techniques. Endoscopic Ultrasound (EUS) and Computed Tomography (CT) are used in identification of pancreatic tumors and in the detection of vascular invasion, thus determining the resectability. To compare the sensitivity and specificity of EUS and CT in the determination of resectability in pancreatic cancer patients, demographic data was gathered from the medical records of patients who presented to MD Anderson Cancer Center from July 1995 to July 2001 with pancreatic cancer. Patients were considered for study that underwent CT scanning, EUS examination and operation. Invasion of vascular structures was measured by the Lawyer grading system and applied to both modalities and compared to operative findings. Data was entered into an Access™ database (Microsoft Corporation, Redmond Washington) created by the investigators. Data was analyzed with a standard statistical software package (SPSS™, Chicago, Illinois). The study is ongoing and no preliminary data is available.

In vitro neopterin levels unaltered in simulated space flight

Mark A. Sultenfuss, The University of Texas Medical School at Houston, Class of 2004

Sponsored by: Anil D. Kulkarni, PhD, General Surgery
Supported by: National Institute of Diabetes and Digestive and Kidney Diseases, T35DK07676
Keywords: immunology, neopterin, space flight, IFNg, cytokines

Culturing cells in a rotating wall vessel (RWV) is a ground-based model used to imitate the physiological changes that occur in space flight. Neopterin, a bi-product of tetrahydrobiopterin production from GTP, is used as a marker for macrophage/monocyte activation and is released in response to IFNg secretion from stimulated T-cells. From this relationship, it can be hypothesized that if IFNg and cell proliferation decrease in simulated microgravity (SMG), then neopterin concentrations should follow, indicating a role in immuno-suppression during space flight. In order to test this hypothesis, neopterin enzyme immunoassays (EIA) and IFNg enzyme-linked immunosorbent assays (ELISA) were utilized to determine their concentrations from supernatants of cells cultured in control tissue culture and RWV for 48 hours. ^{3}H-labeled thymidine uptake was measured to determine cell proliferation. The RWV neopterin concentrations declined by 4.29 percent while IFNg decreased by 70.6 percent and cell proliferation stimulation indices displayed an almost significant decrease ($p = 0.051$). These initial findings suggest that neopterin concentrations are unaltered during SMG and that the suppression of the immune system during space flight may not involve IFNg stimulation of neopterin production by monocytes and macrophages. The next step in the analysis of neopterin involves repetition of this experiment incorporating other ground-based models of space flight, allowing for modification of the hypothesis and improved understanding of the mechanisms involved in immunosuppression and space flight.

MEDICAL SCHOOL

ANESTHESIOLOGY

Validity of somatization measures

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Sponsored by: Diane M. Novy, PhD; Marilu Price, PhD, Anesthesiology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: somatization, measurement, pain

Somatization is the tendency to experience bodily symptoms without organic basis in response to psychological distress. These "unexplained" symptoms are prevalent in health care settings, particularly among patients with pain conditions. In order to provide adequate care for patients with these symptoms, pain physicians need to recognize when the psychosocial aspects of the patient's life are contributing to, or exacerbating, their symptoms. This study examined measurements of somatization. At the time of patients' first appointment at the University Center for Pain Management and Rehabilitation they were approached in the waiting room and asked to complete the Brief Symptom Inventory 18 (BSI 18; comprised of anxiety, depression, and somatization subscales) and a somatization symptom checklist. Following the history and physical examination, the physician reviewed the patient's endorsement of symptoms on the checklist. The physician then checked and rated each endorsed symptom on a scale from 0 to 10 (0 being expected given the patient's diagnosis and 10 being greatly unexpected given the diagnosis). Participants were 97 adults, 54 of whom were male. Average age was 48 years (SD = 14.5 years). Duration of pain ranged from 3 months to 20 years. The majority of participants were white (73.5%), married (55%), and college educated (60%). The results were as expected. Convergent validity was demonstrated by a high correlation ($\underline{r} = 0.74$) between the number of patient reported symptoms and the number of those symptoms the physician reported as incongruent with the diagnosis. The number of patient reported symptoms and the total physician rating was significantly related ($\underline{r} = 0.67$) and gave further support to convergent validity. Additional convergent validity was demonstrated by the significant correlation between the BSI somatization subscale and the number of patient endorsed symptoms ($\underline{r} = 0.62$). Discriminant validity was demonstrated by smaller correlations between the BSI depression and anxiety subscales with patient checklist total and physician somatization ratings. In conclusion, support was found for use of the patient somatization checklist among patients with pain conditions.

A comparison of two different techniques in the instruction of video orotracheal flexible fiberoptic intubation during residency training

Devika Radhika Rao, Trinity University, Class of 2002
Sponsored by: Carin A. Hagberg, MD, Anesthesiology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: orotracheal fiberoptic intubation, video performance

The purpose of this study is to determine if video feedback of the performance of orotracheal fiberoptic intubation by novice anesthesiologists in anesthetized patients is beneficial. Two one-hour lectures will be given about: (1) the anatomy of the upper airway, and (2) the structure, use and care of the fiberoptic bronchoscope followed by a thirty-minute hands-on session with a simulator "Airman" to teach skills in endoscopic manipulation.

The residents will be randomized to be in one of two groups. Both groups will perform ten video-assisted flexible fiberoptic intubations with real-time instruction. All resident performances will be videotaped, and only the group II performances will be reviewed and analyzed prior to the next intubation. Patient subjects will consist of one hundred fasted, healthy (ASA physical status I or II) adult patients undergoing elective surgery requiring tracheal intubation.

Timing of the procedure will begin when the fiberscope is first inserted into the oral cavity and will continue until the first breath is delivered via a successfully placed endotracheal tube (ET). We will time how long it takes to: (1) identify the glottic opening, (2) pass the fiberoptic bronchoscope (FOB) through the glottic opening into the trachea, (3) identify the carina, and (4) pass the ET over the FOB and into the trachea. If tracheal intubation with the FOB is unsuccessful on two attempts or within ten minutes, the attending anesthesiologist will perform direct laryngoscopy. We expect a 30–40% difference in intubation time with the video feedback technique.

BIOCHEMISTRY AND MOLECULAR BIOLOGY

TEA domain subcloning

Raúl Alejandro González, University of Houston, Class of 2002
Sponsored by: Sudha Veeraraghavan, PhD, Biochemistry and Molecular Biology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: subcloning, scalloped, vestigial, TEA domain

In *Drosophila*, the Scalloped protein controls wing development. The Scalloped protein is a selector protein. It binds cis-regulatory elements, DNA sequences near a set of target genes. It is a member of the TEA family of selector proteins. It is understood that Scalloped and the cofactors associated with it regulate expression of the target genes. It was suggested that vestigial protein, naturally present only in wing cells, modulates the conformation of Scalloped allowing it to bind DNA and activate wing morphogenesis. The mechanism of Scalloped function *in vivo* is poorly understood. Progress continues to be made as more research is done pertaining developmental biology. For better understanding of this process and to help elucidate other similar processes, it is important to solve the structure of the 79 amino acid long DNA binding TEA domain, and to understand the interactions between cofactors, selector proteins, and enhancer sites. Also, in order to collect nuclear magnetic resonance data large quantities (about 20–50 mg) of the TEA domain must be produced. Therefore, it is necessary to subclone the TEA domain DNA into bacterial overexpression vectors. The TEA domain construct resulting from this summer research project will be used to make large quantities of protein for structural studies.

Analysis of the modification of amino acids 180 to 547 of the protein 53BP1

Vivian J. Pham, St. Mary's University, Class of 2002
Sponsored by: Phillip Carpenter, PhD, Biochemistry and Molecular Biology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: 53BP1, cell cycle checkpoints, DNA damage

To maintain the genetic stability within a species, the replication of DNA must be efficiently replicated and repaired should any damage occur; otherwise, fatal genetic chaos could occur. To prevent such catastrophes from occurring, checkpoints are established throughout the cell cycle. If there is damage to the DNA, either by UV/γ-radiation or by hydroxyurea, these checkpoints stop the progress of the cell cycle, and repair proteins are activated. From previous experiments, scientists have found a series of signaling proteins that participate in DNA damage response mechanisms. These proteins are phosphorylated at sites in which serine or threonine is followed by glutamine (SQ/TQ sites), and are activated as a result of the phosphorylation. The protein 53BP1 is believed to participate in the DNA damage response pathway. Thus far, research has shown that 53BP1 binds to the tumor suppressor protein p53 (a protein that can activate or repress transcription of genes) in response to DNA damage so as to enhance p53 transcriptional activity. Upon DNA damage, 53BP1 is phosphorylated, although the specific sites of phosphorylation within the region of amino acids 180 to 547 are unknown. In looking at the primary structure of 53BP1 we find that there are numerous SQ/TQ sites. In the previously mentioned portion of 53BP1, there are five sites that could be phosphorylated during DNA damage response. The purpose of the experiment is to determine if these sites are indeed phosphorylated in response to DNA damage. If phosphorylation does take place, we hope to also determine the specific sites of phosphorylation. In order to determine the phosphorylation sites of this small section of 53BP1, a cDNA fragment of human 53BP1 was cloned into a pGEX-4T-1 vector and protein was to be expressed from the cloned DNA. After protein expression, I will purify the material for phosphorylation analysis. Because of limited time constraints, I could not complete this portion of the experiment in time to examine the modifications of 53BP1 in response to DNA damage. Thus, the specific sites of phosphorylation of 53BP1 in this region are still undetermined.

EMERGENCY MEDICINE

The usage of emergency medical services in the pediatric emergency center

Sarita D. Singeetham, University of Texas at Austin, Class of 2004

Sponsored by: Christine E. Koerner, MD, Emergency Medicine
Supported by: The University of Texas-Houston Summer Research Program
Keywords: pediatrics, EMS, ambulances, 911

The primary objective was to determine the characteristics of pediatric patients who present to the emergency department via an ambulance. The secondary objective was to gain an insight into parental perceptions concerning 911 and emergency center utilization.

According to the Medical Director of Emergency Services for the city of Houston there has been a 27% increase in ambulance usage without an overwhelming increase in the acuity of pediatric patients presenting to the emergency center. Although the literature shows that many patients utilize ambulances because they do not have transportation, we believe that this increase may be secondary to a lack of access to primary care.

Our study was performed from July 9 through August 8, 2001 at Lyndon B. Johnson General Hospital, an inner-city county hospital in Houston, Texas. We conducted a survey consisting of a two-page questionnaire administered by an interviewer in English or by an assistant in Spanish. The interview was conducted as guardians presented pediatric patients (ages birth–17years) to the emergency department in the order of their arrival. Patients in critical condition and parents who refused to take part in the survey were excluded from the study. The study population consisted of patients' guardians between the hours of 12 PM and 8 PM from Monday through Friday and 4 PM and 8 PM on Saturday. Questionnaires were not administered on Sunday. Inclusion conditions limited the population to those who accompanied a pediatric patient to the emergency center, accepted participation in the questionnaire, were literate in English or Spanish, and had an intact mental status facilitating their ability to comprehend and respond coherently to an interviewer.

In order to validate the questionnaire, a pilot study was done consisting of 86 patients. The pilot study was conducted for two weeks prior to the actual survey from 9 AM to 5 PM on Monday through Friday. The data from the pilot was not used in the final analysis and was conducted primarily to gain familiarity with the methods of the emergency department and modify inadequate sections of the questionnaire. Pilot data revealed that the questionnaire was adequate with a few negligible changes.

On each day, the interviewer, Sarita Singeetham, was present in the Pediatric Emergency Department and asked consecutive guardians to participate in the questionnaire after giving verbal informed consent. The guardian was never required to record responses single-handedly as the interviewer marked each response.

The questionnaire consisted primarily of three sections as follows: demographic and basic information, past history and current information, and a scenario section. Demographic data included age, sex, and ethnicity of the patient, age, educational level, and relationship of the caretaker, method of payment, and the chief complaint. The second section customarily included questions in an effort to gain information concerning the patients past medical history. These questions included the presence and type of current physician for the patient, the physician's response to the guardian's concerns prior to presentation to the emergency department, patient immunization data, and history regarding previous presentation to the emergency department. The third scenario section of the questionnaire was composed of ten scenarios, some of which involved a laceration, foreign body, or toxic ingestion. The interviewer read a short scenario with reference to a dilemma for the guardian and the guardian was asked two questions: first, "Would you call 911" and second, "Would you take the child to the Emergency Center." This section was utilized to provide us with a better perspective with respect to parental perceptions pertaining to 911 usage and the capabilities of the emergency department. The interviewee responded on a scale of 1 to 5, either strongly agreeing with the proper use of calling 911 or going to the emergency center with a response of five or a 1 for strongly disagreeing with calling 911 and using the emergency department. Finally, additional comments were documented and the final diagnosis of the patient was verified in order to conduct a post-interview evaluation.

330 interviews have been conducted thus far, and I plan to continue interviewing until August 8, 2001. The data will be statistically analyzed and conclusions will be drawn establishing a foundation for further studies.

INTEGRATIVE BIOLOGY AND PHARMACOLOGY

Novel Regulators of Soluble Guanylyl Cyclase

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Sponsored by: Emil Martin, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: soluble guanylate cyclase, protein purification, enzyme activity

Soluble guanylate cyclase (sGC) is an enzyme that converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP). cGMP is an intracellular messenger molecule affecting a variety of cellular functions, including smooth muscle relaxation, neurotransmission, platelet aggregation, etc. Addition of nitric oxide (NO) donors such as sodium nitroprusside (SNP) or allosteric regulators such as YC-1 have been shown to increase sGC activity. One important avenue of research is the search for novel *in vivo* regulators of sGC. A protein library was screened using yeast two-hybrid approach. Several previously identified positive clones were subcloned into a pGEX vector and transformed into BL21 strain of *E. coli*. The clones were fused in frame to glutathione S-transferase (GST). After the GST-fusion protein was overexpressed, it was purified by affinity chromatography on glutathione-sepharose column. The proteins were eluted with 15 mM glutathione. The proteins, named #4, 5, 9, and 37, were incubated with lysates of a BE2(C) cell line, which expresses sGC. The effects of these proteins on basal, SNP- and YC-1 activated sGC were tested. It was found that all proteins inhibited sGC activity, but with different specificities (e.g., #37 inhibited the basal levels 10-fold, while others inhibited the basal activity only 3-4 fold). All proteins inhibited SNP activation about 2-fold, while YC-1 stimulation was attenuated 1.6-2 fold. Further studies should test the direct binding of these proteins to sGC and examine their effect on activity of purified sGC and their mechanism of action.

NSAIDS contribute to bile salt-induced apoptosis of HepG2 cells

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Sponsored by: Lenard Lichtenberger, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: NSAIDs, PC-phosphatidyl choline, BS-bile salts, GI gastroenterologic

In a world with an increasingly aging population, NSAID use is a daily occurrence. GI side effects of these drugs has become the focus of much research. Many factors contribute to the toxic effects of NSAIDs. In normal human physiological conditions, phospholipids protect the epithelium of the GI tract from the toxic effects of noxious agents present, such as HCl or bile salts. Phosphatidyl choline, a phospholipid, protects the intestinal and gastric mucosa against the injurious action of bile salts, possibly by forming less toxic mixed micelles. When an NSAIDs is introduced into the GI tract, it damages the mucosa not by direct action but by possibly competing for the available protection of PC molecules. This mechanism allows the hydrophobic protective barrier of stomach epithelial cells to become wettable, which allows gastric acid or bile to become in close contact and let its corrosive effects occur. In past studies the pairing of NSAIDs with a phospholipid was proven to increase protection against GI ulceration and bleeding and increases efficacy as an anti-inflammatory and anti-pyretic. GI bleeding and lesions dramatically decreased if a phospholipid paired with NSAIDs during treatment. A second mechanism suggests that bile salts become toxic and cause apoptosis by injury, if NSAIDs are introduced. In an *in vitro* model, HepG2 or human hepatoma cells were utilized in testing this hypothesis. The projected experiment involves the growth and maintenance of a HepG2 cell line. This cell line will be treated with NSAIDs only, BS only, BS and NSAIDs, or BS, NSAIDs, and PC. A control group of untreated cells will also be maintained for comparison. After treatment, the cells will be analyzed for apoptosis by four methods of measurement: DNA laddering, *in situ* nick end labeling, western blot utilizing PARP (an apoptotic enzyme), and an Annexin V kit. The results are expected to show an increased level of apoptosis in the BS and NSAID treated cells, and a decreased level of apoptosis in the cells supplemented with PC in addition to BS and NSAIDs.

Protective effect of lactoferrin on acute intestinal bleeding induced by indomethacin and L-NAME in the rat

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Supported by: The University of Texas-Houston Summer Research Program; Agennix Inc. of Houston
Keywords: lactoferrin, indomethacin, NSAIDs

Indomethacin and other non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to reduce inflammation, pain and fever. However many people who use these drugs suffer severe gastrointestinal side effects, such as intestinal bleeding and peptic ulcers. To counter-act these effects, researchers are investigating the protective benefits of lactoferrin, a natural glycoprotein. Lactoferrin has antibiotic and anti-inflammatory properties that protect the gastrointestinal tract from chronic (four-day) indomethacin damage. This study was designed to determine the effectiveness of lactoferrin on acute (one-day) indomethacin-induced intestinal bleeding. The control groups were dosed with bovine serum albumin (BSA), while the experimental groups were administered lactoferrin (200mg/kg) orally or intraperitoneally. Both lactoferrin groups and one BSA group were then administered indomethacin (10mg/kg) to induce intestinal bleeding and three doses of L-NAME (20mg/kg), a nitric oxide synthase inhibitor, to enhance the damage throughout the day. Twenty-four hours after the initial dosing, the animals were euthanized. Intestinal washes were taken for hemoglobin analysis and intestinal tissue samples were taken for myeloperoxidase, an index of inflammation. Hemoglobin analysis indicated that both lactoferrin-treated groups had lower levels of intestinal bleeding (oral = 84.4%, IP = 57.1% reduction) compared to the BSA/Indomethacin group without lactoferrin ($p < 0.05$). Myeloperoxidase analysis will be conducted on the tissue samples to determine the location and severity of bleeding along the intestine. This experiment determined that lactoferrin can significantly reduce acute intestinal bleeding induced by indomethacin and L-NAME. Future experiments should be conducted to understand the mechanism by which lactoferrin prevents NSAID-induced intestinal damage.

The effects of nerve injury on inhibitory synaptic potentials in *Aplysia* sensory neurons

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Sponsored by: Edgar T. Walters, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, NS35979
Keywords: nerve injury, hyperpolarization, inhibitory post-synaptic potential, excitability

Sensory signals are communicated by action potentials in sensory neurons (SNs), which are initially elicited by depolarization of the cell's negative resting potential. Localization of sensation is enhanced by excited SNs hyperpolarizing surrounding SNs through inhibitory interneurons. Although nerve injury produces hyperexcitability of sensory neurons, an unanswered question is whether injury affects hyperpolarization. I hypothesized that hyperpolarization of injured cells would be greater than in control cells. Pedal nerves on one side of *Aplysia* were crushed, the animals were sacrificed four days later, and the pedal-pleural ganglia were removed, along with the P9 nerves. The nerve threshold was defined as the current needed to produce an action potential in a P9 SN during a 5ms nerve shock. SNs were then tested for excitability by recording the number of action potentials fired during a 2nA 2s intracellular pulse, and the hyperpolarization elicited by four 5ms nerve pulses at 3 times nerve threshold was recorded. As predicted, the crush cells, which fired a mean of 22.7 action potentials, were significantly more excitable than the control (4.8 action potentials) and naïve cells (3.8 action potentials; $p < 0.0001$; ANOVA and Newman-Keuls's). In addition, the crush cells showed a significantly greater hyperpolarization (-6.9mV) than the control and naïve cells did (-2.0mV; $p < 0.0001$). In general, excitability and hyperpolarization were significantly correlated at $r = -0.53$ ($p = 0.0001$). These results raise interesting questions about the loci and mechanisms of enhanced inhibitory input to SNs after nerve injury.

Ibuprofen vs. Ibuprofen/PC treatment for peripheral neuropathy in the rat model

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Sponsored by: Lenard M. Lichtenberger, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, DK53195
Keywords: Ibuprofen, phosphatidylcholine, hyperalgesia, analgesic, neuropathy, sciatic nerve

Research in our laboratory has demonstrated that the therapeutic activity of nonsteroidal anti-inflammatory drugs (NSAIDs) to inhibit fever, inflammation and pain, can be enhanced if these drugs are associated with phosphatidylcholine (PC). However, effects of PC-NSAIDs on peripheral neuropathy have yet to be investigated. Using sixteen male Sprague-Dawley rats, recovery surgery was performed to expose the sciatic nerve. The left leg was used for sham surgery. The right leg, after isolating the sciatic nerve, had four loose ligatures of chromic gut tied around it to cause inflammation and thus hyperalgesia. After three days, animals were given doses of Ibuprofen, Ibuprofen/PC, or saline (control) by oral gavage. Behavioral tests were performed to determine the animal's sensitivity to pain. Von Frey Hairs, which bend at a given pressure, were repeatedly pushed against the hind paws of the animals and the number of paw withdrawals were recorded as an index of pain. A second test measuring pain through thermal stimulation was used to measure latencies of paw withdrawal. The raw data showed the expected trend that Ibuprofen/PC would be a better analgesic than Ibuprofen alone, though the differences were not statistically significant. In conclusion, Ibuprofen/PC has the tendency to have greater analgesic properties than Ibuprofen against inflammation-induced neural pain and more research needs to be conducted to assess the significance of this effect.

Expression and location of the LAR PTPase receptor in MCF-7 cells

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Supported by: The University of Texas-Houston Summer Research Program; Army, DAMD17-00-1-0460
Keywords: breast cancer, diabetes, phosphatase

Diabetic women have a greater risk of developing breast cancer. The leukocyte common antigen-related (LAR) protein tyrosine phosphatase (PTPase) is a possible link between diabetes and breast cancer. The presence of LAR in the focal adhesion zones of breast cancer cells is associated with increased metastatic growth of the cells. Also, the PTPase intracellular domain of LAR dephosphorylates the activated insulin receptor thus inactivating the insulin receptor. In order to determine the effect of estradiol (E2) on the level of expression of LAR, MCF-7 breast cancer cells were subjected to E2 for up to four hours. These cells were then extracted and subjected to immunoblot analysis where they were probed for the extracellular and intracellular domains of LAR. Results show that expression of the extracellular domain of LAR decreases 50% within the first 30 minutes of incubation, whereas the intracellular domain remains constant throughout the 4 h incubation. This indicates that the extracellular domain is shed into the cell culture medium upon E2 stimulation. The effect of E2 on the subcellular location of LAR was then determined. MCF-7 cells were subjected to E2 incubation for two hours and then processed to recover the plasma membranes and endosomes of the cells. These extracts were then subjected to immunoblot analysis where they were probed for the extracellular and intracellular domains of LAR. Results from this experiment were inconclusive. Future experiments will assess the expression of LAR in breast cancer cell growth.

Long term and short term effects of heat stress in *Aplysia*: A behavioral and electrophysiological study

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Sponsored by: Edgar T. Walters, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, NS35882
Keywords: reflex threshold, withdrawal response, cellular threshold, heat shock proteins

Heat and other stresses induce the expression of heat shock proteins(HSP), which are thought to protect neurons from subsequent stresses. An interesting question is whether HSPs are involved in the electrophysiological changes that result from stress. The goal of this study was to examine the long-term and short-term behavioral and electrophysiological responses to heat shock in the sea mollusk *Aplysia*. For each test animal, a behavioral test was followed by an electrophysiological test. I did the behavioral tests while Andrea Pingitore made the intracellular recordings. In the behavioral tests, changes in excitability and reflex thresholds were monitored 1 hour and 24 hours after heat shock. The heat shock treatment consisted of placing the animal in 30°–35°C water for up to 40 minutes. Untreated control animals were also tested. After an animal was observed at 24 hours, the animal was dissected, the pedal-pleural ganglia were removed, and intracellular recordings were conducted, 1–2 hours later, in the sensory neurons. Intracellular recording were immediately conducted in one ganglion. Simultaneously, the other ganglion was administered the same heat shock that the live animal had received before being sacrificed. Intracellular recordings of the twice-heated ganglion were made approximately one hour after the dissection. The behavioral results showed a reduction in excitability, which was present, both 1-hour and 24 hours after heat shock. There was also a long-term elevation in reflex threshold. Similarly, the intracellular recordings showed a reduction in excitability, as well as, an elevation in cellular threshold. The test cells were significantly less excitable than the control cells and the twice-heated cells were less excitable than the once- heated cells. These observations set the stage for more detailed investigations on the functions and mechanisms of long-lasting behavioral and sensory responses to heat shock.

Degradation of $ß_1$-tubulin in CHO cells by the ubiquitin-proteasome pathway

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Sponsored by: Fernando R. Cabral, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, RO1 CA85935
Keywords: $ß_1$-tubulin, ubiquitin, proteasome, lactacystin

Degradation of cellular proteins is an important process in the life cycle of a cell. Degradation must occur to remove damaged or mutated proteins, to regulate the cell cycle, and to maintain normal cellular physiology. The ubiquitin-proteasome pathway plays a key role in this degradation, helping the cell to maintain precise steady-state concentrations of proteins and rid itself of proteins that have lost function. In CHO cells, $ß_1$-tubulin has a long half-life, remaining virtually undegraded. Assembly defective, mutant tubulin, however, is rapidly degraded. Additionally, $ß_1$-tubulin subunits expressed in excess of α–tubulin are also rapidly degraded. It was hypothesized that the ubiquitin-proteasome pathway degrades these proteins. Strain 6H2, expressing assembly defective $ß_1$-tubulin, and WTHA ß1#4 cells, overexpressing wild type $ß_1$-tubulin, were treated with lactacystin overnight to stabilize the protein targeted for degradation. Protein samples were prepared from these cells, run on SDS-PAGE gels, and blotted with antibodies to $ß_1$-tubulin and actin as a control. Chemiluminescence and chemifluorescence were used to visualize and quantify the protein bands. The results did not support the hypothesis that the $ß_1$-tubulin was degraded by the ubiquitin-proteasome pathway. Further study should include the use of a positive control for lactacystin and the investigation of different proteasome inhibitors.

Effect of the mouse LXR nuclear receptor on the expression of rabbit Cyp7A

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Sponsored by: David Loose-Mitchell, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: HepG2, Cyp7A, LXR, luciferase (Luc)

The LXR nuclear receptor is a mouse hepatic cell. It binds with RXR as a heterodimer and this complex then binds to the Cyp7A promoter. Upon binding and being stimulated by a ligand (25 hydroxy cholesterol used in this experiment), this complex has been demonstrated to increase the transcription of the Cyp7A promoter in mice. My study was to see if this same mouse LXR nuclear receptor would bind to the rabbit cyp7A promoter and have the same effect as it did in mice, or if it would not stimulate transcription, as is the case in humans. DNA constructs made by previous coworker Ruth Ann Buck, PhD, were used to test transcription activity. These constructs contained the rabbit Cyp7A promoter and the gene for Luciferase. The LXR and rabbit DNA contructs were transfected into human HepG2 cells (cancerous liver cells) and data was collected using Promega's Luciferase Assay kit. The data showed that upon the addition of ligand by itself, there was an increase in the transcription of Luciferase suggesting that there was sufficient endogenous LXR to stimulate Cyp7A expression. In fact, after the addition of the LXR plasmid, the transcription decreased. An explanation for this decrease is the possible presence of inhibitory regions in the full-length promoter.

Characterization of intrinsic GTPase activity of Rac1b

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Supported by: The University of Texas-Houston Summer Research Program
Keywords: Rho family, small G protein, Rac1b, GTPase activity

Rac1b is a member of the Rho family of small GTP-binding proteins. Rac1b serves as a molecular switch, which is regulated by guanine-nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs) to alternate between an inactive GDP-bound form and an active GTP-bound form, respectively. Known to be involved in the formation of lamellipodia and membrane ruffling, Rac1b is a splice variant of Rac1 that may be overexpressed in various cancers. To begin to understand the GTPase activity of Rac1b, the intrinsic GTPase activity of Rac1b was compared to that of wild-type Rac1. Intrinsic GTPase activity was measured by preloading GST, GST-wild-type Rac1, GST-Rac1b, and GST-V^{12}Rac1 with [γ^{32}P]GTP, with GST serving as a negative control. V^{12}Rac1 contains a mutation that inhibits its intrinsic GTPase activity and thus serves as a secondary control. After incubation, the preloaded proteins were added to cold assay buffer containing excess $MgCl_2$. After initial samples were collected at the zero time point, the remaining protein was incubated at 20°C. Subsequent samples were removed at 3-, 5-, 10-, and 15-minute intervals and diluted into cold assay buffer. The samples were filtered through nitrocellulose filters, and the amount of [γ^{32}P]GTP-bound protein bound to the filters was determined by liquid scintillation. The results of the GTPase assay indicate that the rate of intrinsic GTPase activity of Rac1b is significantly greater than that of wild-type Rac1.

INTERNAL MEDICINE

Effects of CD1E genes on arteriosclerosis

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Sponsored by: Yong-Jian Geng, MD, PhD, Internal Medicine—Cardiology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: CD1E, polymorphism

CD1 molecules are structurally and functionally similar to major histocompatibility complex class 1 molecules. It presents lipids or glycolipids to T cells. The CD1e may be functionally significant considering the nature of the amino acid substitutions and the frequency of allelic forms in the human population. Atherosclerotic lesions consist of T cells, CD1 + macrophages, and lipid accumulation. T cells bind to lipid antigen presented by CD1, which cause autoimmune response.

The main purpose of this research is found out about any involvement of CD1E in autoimmune disease such as arteriosclerosis, scleroderma, and SLE. PCR specifically amplify the Exon 2. CD1E polymorphism was found at 79 amino acid positions. A nucleotide changed from A to G resulting in an amino acid replacement of glutamine to arginine. These give two different alleles – allele a and allele b. The enzyme that we used to digest CD1E was ACC III that cuts only b alleles. That way we are able to distinguish between a and b. The DNA that have two fragments have both a and b alleles. The DNA that has only one fragments is either homozygous alleles a or alleles b.

The DNA digestion by the restriction enzyme ACCIII gives allele frequencies of the patients. We are able to find the pattern of frequencies and determine how the alleles are associated with the other autoimmune disease.

Preventing cardiovascular disease in the city of Houston

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Sponsored by: Francisco Fuentes, MD, Internal Medicine—Cardiology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: cardiovascular disease, preventing

Cardiovascular disease is one of the leading causes of death in the United States. Therefore, trying to prevent its occurrence in the city of Houston by implementing educational programs on cardiovascular disease in the 88 super neighborhoods would decrease its incidence. An ongoing project on the website *www.houstonhearthealth.com*, will allow the public of Houston to click on their designated neighborhood and obtain valuable information on cardiovascular disease and prevention. Different clinics as well as citywide programs on smoking cessation were obtained to add to the different neighborhoods. The prevalence of certain risk factors in the first 29 neighborhoods in Houston was updated with data from the 2000 census. In an effort to decrease the incidence of cardiovascular disease in the city of Houston, educational programs can be held at neighborhood community centers as well as clinics in order to expose the public to disease prevention. In addition, making certain that methods used to prevent the disease as well as its risk factors are addressed in public advertising would also aid in decreasing the prevalence of cardiovascular disease.

DNA sequencing of selectins P, E, and L as candidate genes for the novel bleeding disorder, East Texas type

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Supported by: The University of Texas-Houston Summer Research Program
Keywords: bleeding disorder, DNA sequencing, selectins

A novel bleeding disorder characterized by easy bruising and life-threatening bleeding with trauma has been identified in a large east Texan family. Through linkage analysis, the disease locus has been mapped to a 1.25 Mb region on chromosome 1q23 which contains 8 known genes. Clinical analysis indicates that the defective protein causing the bleeding disorder could be a soluble protein in plasma or a protein that modifies a soluble protein. Therefore, the secretory proteins (selectins E, P, L and SCYC 1,2), which are located within the critical region, are possible candidates for the defective gene causing this bleeding disorder. The hypothesis to be tested is that a mutation in selectins E, P, or L disrupts the coagulation cascade leading to bleeding disorder. Intron based exon specific primers were designed, PCR was performed to amplify the genes from proband sample, product was purified and sequenced using ABI3700 automated sequencer. No disease causing mutations were found in the sequences analyzed, thus further investigation will be needed. Identification of the defective gene will lead to better understanding of the disorder and the advancement in the study of coagulation cascade and thrombosis.

Expression and purification of modified human cytochrome P450 1A2 in *Escherichia coli*

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Sponsored by: Lee-Ho Wang, PhD, Internal Medicine—Hematology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, HL-60625
Keywords: cytochrome P450 1A2, *Escherichia coli*, heterocyclic amine, carcinogenic

Human cytochrome P450 1A2 is the enzyme involved in the metabolism of a variety of compounds, such as caffeine, and in the conversion of heterocyclic amines into their carcinogenic forms. This enzyme, located at the endoplasmic reticulum, requires the ubiquitous P450 reductase to provide the electrons necessary for catalysis. In order to better understand the interaction between P450 1A2 and reductase, we transformed *Escherichia coli* with trc promoter-driven vector containing P450 1A2 cDNA modified by a $(histidine)_5$ tag insertion at the carboxyl terminus. Centrifugation removed cell debris, and ultra-centrifugation was employed to pellet the membrane fraction. The protein concentration of the membrane fraction was found to be 10.7 mg/ml. A final concentration of 2.0 mg/ml was reached and P450 1A2 was solubilized overnight using 1.0% CHAPS in a potassium phosphate buffer. The solubilized material was loaded onto an equilibrated Ni^{2+} nitrilotriacetate column. Extraneous matter and potential contaminants were removed with an equilibration and a 20mM imidazole buffer. The enzyme was eluted with a 300mM imidazole buffer. With the use of a spectrophotometer, we realized that the enzyme was not released in the 300mM imidazole wash. After determining the cause and repeating the experiment, the P450 concentration will be assessed by Fe^{2+} • CO versus Fe^{2+} difference spectroscopy.

The pattern of VEGF expression during HSC activation in response to hypoxia in stellate cells

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Supported by: The University of Texas-Houston Summer Research Program
Keywords: VEGF expression, stellate cells, EPO, neovascularization

The overall objective is to investigate the pattern of VEGF expression during hepatic stellate cells activation in response to hypoxia. A central event in the pathogenesis of tissue fibrosis is the activation of resident perivascular mesenchymal cells, which proliferate and produce extracellular matrix in response to injury. In the liver, the hepatic stellate cell (HSC) also known as lipocyte fulfills this role. The hepatic stellate cell is analogous to the kidney's oxygen sensing cell, the renal fibroblast-like cell in the fact that both cell kinds express the erythropoietin (EPO) gene when subjected to hypoxia. Vascular endothelial growth factor (VEGF), a key mediator in angiogenesis is a highly conserved disulphide bonded dimeric glycoprotein that exists as one of four different isoforms. This multifunctional cytokine is associated with angiogenesis also known as Neovascularization.

This investigation carried out showed increased cellular proliferation and vascular permeability as a result of VEGF expression and erythropoietin production when fresh cultured stellate cells obtained from the liver of rats in the laboratory were subjected to hypoxia using a gaseous composition of 94% Nitrogen, 1% Oxygen, and 5% Carbon dioxide. The adaptive responses, a critical requirement of all living organisms, serve to increase oxygen delivery or provide alternative metabolic pathways that do not require oxygen.

This procedure was carried out utilizing the livers of two rats after passing different digestive enzymes through their capillary beds like Collagenase and DNase and having cleaned them with high and low Pronase solutions using their hepatic portal veins and inferior vena cava as ports.

Tumor necrosis factor-α decreases metabolic gene expression in the rat heart

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Sponsored by: Heinrich Taegtmeyer, MD, DPhil, Internal Medicine—Cardiology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, RO1 HL/AG 61483
Keywords: tumor necrosis factor-α, cardiac metabolism, heart failure, gene expression

The failing human heart has been described as energy starved. The expression of key regulators of myocardial energy substrate metabolism is severely depressed in the failing heart, suggesting a possible transcriptional mechanism for energy starvation. Previous studies have implicated a rise in the cytokine tumor necrosis factor-α (TNF-α) in the pathogenesis of heart failure. We therefore examined the hypothesis that TNF-? may play a role in energy starvation of the failing heart at a transcriptional level. Eleven male Sprague-Dawley rats were injected with either TNF-α (30μg/kg: tail vein) or saline. Twelve hours later, hearts were isolated and analyzed using real time quantitative RT-PCR for mRNAs encoding for key regulators of glucose utilization, fatty acid oxidation, as well as contractile proteins. These included glucose transporters 1 and 4 (GLUT1 and GLUT4), pyruvate dehydrogenase kinases 2 and 4 (PDK2 and PDK4), citrate synthase (CS), muscle carnitine palmitoyl transferase (mCPT1), malonyl-CoA decarboxylase (MCD), medium chain acyl-CoA dehydrogenase (MCAD), long chain acyl-CoA dehydrogenase (LCAD), myosin heavy chain isoforms α and ß (MHC-α and MHC-ß), and cardiac and skeletal α-actin isoforms. Of these, GLUT4, MCD, MCAD, LCAD, PDK2, CS, MHC-α and cardiac α-actin were all decreased ($p < 0.05$), while GLUT1, PDK4, mCPT1, MHC-ß, and skeletal α-actin were not significantly altered in response to TNF-α treatment. In conclusion, treatment of rats with TNF-α mimics many of the changes in gene expression observed in the failing human heart. These observations are consistent with the hypothesis that TNF-α induces energy starvation.

Effects of double knockout CD1-ApoE genes on arteriosclerosis

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Sponsored by: Yong-Jian Geng, MD, PhD, Internal Medicine—Cardiology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, HL59249
Keywords: arteriosclerosis, gene knockout, immunohistochemistry

It was evident that arteriosclerotic lesions were expected in the ApoE knockout mice. CD1 is a membrane protein involved in lipid antigen presentation. We now wanted to see the correlation of the double knockout genes and effects on arteriosclerosis as compared to the visible signs and symptoms of arteriosclerosis in the ApoE Knockout mice or wild type controls.

The mice were given a high cholesterol diet to induce early signs of arteriosclerosis. Once they were sacrificed, their hearts taken and cut and made into frozen sections and transferred onto slides. The tissue would be embedded in OCT Compound. In -20°C temperature, the OCT Compound gel hardens and surrounds the tissue completely creating an OCT Block, and a cross section of the heart and the aorta is made with the cryostat machine (frozen sections can be made with a thickness of 6 microns). Once sections are made they are properly stored in -20°C freezers for later studies.

Single and double staining was done to the frozen sections. Changes in incubation time, antibody concentration, antibody host, incubation temperatures were made to see difference in specific staining rather than the non-specific staining.

In conclusion, we did get a perfect staining and were able to see the specific-binding staining. We were able to confirm this by pulling published articles in which the same antibody was used on cardiac tissue. The pictures that were illustrated in the article were similar to our findings.

Incidental therapeutic drug monitoring (TDM) of protease inhibitors (PIs) as surrogate marker of non-adherence to antiretroviral therapy in HIV positive patients

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Sponsored by: Roberto Arduino, MD, Internal Medicine—Infectious Diseases
Supported by: The University of Texas-Houston Summer Research Program
Keywords: therapeutic drug monitoring, adherence

Non-adherence to antiretroviral therapy has been shown to allow for increased viral replication and mutations leading to resistance to antiretroviral therapy in HIV/AIDS patients. In patients receiving antiretroviral therapy for the treatment of HIV/AIDS, there is a need for a method to accurately assess patient adherence in order to increase adherence and decrease viral resistance to antiretroviral regimens. The current standard for the assessment of adherence is patient self-report which is neither accurate nor reliable. One proposed method, therapeutic drug monitoring (TDM), measures the plasma drug concentrations of certain antiretroviral medications used in the treatment of HIV/AIDS. Currently this testing is available only for the protease inhibitors (PI) lopinavir, indinavir, nelfinavir, saquinavir, and amprenavir. To determine whether TDM is an assessor of non-adherence in patients on antiretroviral therapy, plasma drug concentrations were measured in patients failing a PI containing regimen. These levels were also measured in patients of the same sex, body mass index, and PI who were not failing. Patient self-report, Physician questionnaire, and pharmacy refill checks were also used to evaluate patient adherence for comparison with TDM results. Due to inadequate data based on difficulty finding patients of this population, further research will be required to draw any conclusions on the use of TDM to determine non-adherence in HIV/AIDS patients.

MICROBIOLOGY AND MOLECULAR GENETICS

Analysis of *Myxococcus xanthus* gliding motility

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Sponsored by: Heidi B. Kaplan, PhD, Microbiology and Molecular Genetics
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, GM47444
Keyword: *Myxococcus xanthus,* gliding motility

Myxococcus xanthus, a gram-negative bacterium, migrates across solid surfaces using gliding motility. Gliding motility consists of single-cell movement termed adventurous (A) motility and group movement termed social (S) motility. Mutagenesis experiments have shown that Type IV pili, lipopolysaccharide (LPS) O-antigen and fibrils are all required for social motility, whereas very little is known about the requirements for A motility. I have studied a variety of gliding motility mutants to learn more about *M. xanthus* surface translocation. Both wild-type and mutant cells were grown overnight in liquid culture, spotted onto nutrient-agar covered microscope slides, and covered with a glass coverslip to provide an agar to solid-surface interface to enhance gliding. The slides were placed at room temperature for three hours before microscopic examination. Ten individual cells of each type were tracked for thirty minutes. DK1218 (A^-S^+) cells maintained a shorter distance between cells then wild-type (an average 1.25 mm compared to 4.73 μm for wild-type). DK1300 (A^+S^-) cells glided for approximately three times longer then wild-type (an average of 7.05 min compared to 1.97 min for wild-type). Surprisingly, HK2001 (A^-S^-) cells, which were expected to be non-motile, did move. However, they remained stationary for periods longer then wild-type (an average of 2.8 min compared to 1.8 min for wild-type). These results support the popular notion that cell proximity is necessary for S motility, but also raise questions concerning our current understanding of gliding motility. Our results showing that A^-S^- cells are motile suggest the active participation of other factors such as LPS O-antigens and fibrils in *M. xanthus* gliding motility.

Targeted and random mutagenesis of the visual pathway of *Chlamydomonas reinhardtii*

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Sponsored by: John L. Spudich, PhD, Microbiology and Molecular Genetics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: rhodopsin, plasmid transformation, selection

The green alga *Chlamydomonas reinhardtii* has been of particular interest in the field of cell biology because light detected by visual pigment-like receptors in its primitive "eye" induces an action potential resulting in photomotility response. The objectives of this project are to identify the components of the photosignaling pathway and to knock-out two rhodopsin-encoding genes we have recently identified to elucidate their role in controlling the cells' motility. First, the selection of photoresponse-negative mutants was designed by a capillary selection technique. However, the transformation efficiency of electroporation has to be optimized in order to produce a random mutation library. Plasmid-mediated insertional mutagenesis is being tested with varying linearized plasmid concentrations (1–16 μg) and different voltages (1800–3000 V/cm). Second, a new vector for knock-outs of specific genes by homologous recombination has been designed and constructed by combining two independent plasmids, which contain spectinomycin and bleomycin resistant genes. We have constructed the vector by recombinant DNA techniques involving restriction digestion, DNA fragment isolation, and ligation. The next step is to introduce upstream and downstream regions of the targeted rhodopsin genes.

This project is under the supervision of Dr. Kwang-Hwan Jung in Dr. Spudich's laboratory.

Disruption of a *Dictyostelium discoideum* gene encoding a homolog of human G protein-coupled gamma-aminobutyric acid receptors

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Sponsored by: Dale Hereld, MD, PhD, Microbiology and Molecular Genetics
Supported by: The University of Texas - Houston Summer Research Program
Keywords: *Dictyostelium discoideum*, gamma-aminobutyric acid receptors, G protein-coupled receptors

Dictyostelium discoideum, a soil amoeba utilized in studies of motility, signal transduction, and development, relies on secreted signaling molecules and receptors for intercellular communication. These pathways enable *Dictyostelium* to aggregate, form multi-cellular fruiting bodies, sporulate, and survive starvation. The emerging genome sequence of *Dictyostelium* has revealed at least 7 homologs of human G protein-coupled receptors for γ-aminobutyric acid (GABA), an inhibitory neurotransmitter. To understand the *Dictyostelium* GRL (for "GABA receptor-like") functions, we undertook a gene disruption approach. We chose to knockout GRL5 because it is the sole type 1 receptor and, by analogy with mammalian GABA receptors, potentially required for the remaining 6 GRLs to function. The GRL5 sequence was amplified by polymerase chain reaction (PCR) and subcloned into Bluescript (pBS). A selectable marker for Blasticidin S resistance was ligated into a unique site within the GRL5 sequence. The resulting knockout construct was released from pBS by cutting flanking restriction sites and transformed into wild type *Dictyostelium* cells by electroporation. The cells were immediately separated into 24 pools ensuring that independent clones could be analyzed. After 24 hours, the medium was supplemented with Blasticidin S. Clones surviving the selection were isolated and assayed by PCR to identify those that incorporated the knockout construct into the GRL5 gene. After verifying their genotypes by Southern blotting, the mutant cell phenotypes will be compared to wild type cell phenotypes to gain insight into the function of GRL5 in *Dictyostelium* development and cell-to-cell communication.

The effect of a putative carbonic anhydrase gene, ytiB, on toxin gene expression by *Bacillus anthracis*

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Sponsored by: Theresa M. Koehler, PhD, Microbiology and Molecular Genetics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: Bacillus anthracis, CO_2, toxin production, carbonic anhydrase

Bacillus anthracis is a gram-positive sporulating bacterium that causes anthrax. *B. anthracis* virulence factors include a poly-D-glutamic acid capsule, and three toxin proteins: edema factor (EF), lethal factor (LF), and protective antigen (PA). Binary combinations of the toxin proteins comprise edema toxin (EF and PA) and lethal toxin (LF and PA). Transcription of the EF, LF, and PA genes is increased during growth of *B. anthracis* in elevated CO_2 (5% or greater). The "CO_2 effect" has significance for a mammalian pathogen. We identified a putative carbonic anhydrase gene (*ytiB*) in the unfinished sequence of the *B. anthracis* genome. Carbonic anhydrases catalyze the reaction $CO_2 + H_2O \leftrightarrow HCO_3 + H^+$. We hypothesized that *ytiB* plays a role in the CO_2 effect. A *ytiB* deletion mutation was introduced into strains harboring transcriptional fusions between the PA gene (*pagA*) and LF gene (*lef*) promoters and the ß-galactosidase gene, *lacZ*. Similar strains were constructed harboring deletions of *ytjA*, a highly-conserved gene located adjacent to *ytiB*, and in the opposite orientation. Enzyme activity was measured during growth in 5% CO_2. The mutant and parent strains did not differ significantly in toxin gene expression or growth rate. These results indicate that either: (1) the products of the *ytiB* and/or *ytjA* genes are non-functional; (2) *ytiB* and *ytjA* do not have a role in the CO_2 effect; or (3) an alternate pathway exists for CO_2-induced toxin gene expression, perhaps involving other carbonic anhydrases or regulatory proteins.

NEUROBIOLOGY AND ANATOMY

Modulation of place cell firing rates by apparatus translation

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Sponsored by: James J. Knierim, PhD, Neurobiology and Anatomy
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, NS39456
Keywords: hippocampus, place fields, firing rates, gain fields

The rat hippocampus is crucial for navigation and spatial learning (O'Keefe and Nadel, 1978). Hippocampal pyramidal cells selectively fire in specific locations of an environment, and may be the neural substrate for cognitive maps. Recent experiments have shown that place fields are tied to a local reference frame—the behavioral apparatus—when the apparatus is moved in a global reference frame (Knierim, unpublished 2001). In the visual system, posterior parietal neurons transform retinotopic locations into global coordinate frames by the product of a gain factor that is a function of the position of the eyes in the orbits (Andersen et al. 1985). We tested whether hippocampal cells transformed a spatial representation from a local to global framework by a similar mechanism. Place cell firing rates were recorded from 4 rats running on a circular track (30" diam.) or on a rectangle (18" x 24") placed in the center, east, or west location of a room. We proposed that for place cells that fired most strongly at the west side of the room, their firing rates at the center location would be greater than the firing rates at the east, and conversely, for cells that fired most at the east location, the firing rate at the center would be greater than at the west. In addition, we predicted that the firing rates among the translations could be described by a gain factor that is a function of apparatus location. The mean peak firing rates for cells strongest at the east were: east: 5.95 (± 0.92), center: 4.73 (± 0.84), and west: 4.21(± 0.77), hertz. For cells strongest at the west, the mean peak firing rates were: west: 5.70 (± 0.84), center: 4.06 (± 0.72), east: 3.97 (± 0.62), hz. The differences in peak firing rates were not statistically different. Thus, these data provide no evidence that the hippocampus transforms a local coordinate frame into a global frame by modulating firing rate as a function of local frame location.

Synaptic connections of an ON parasol ganglion cell in the macaque retina

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Sponsored by: David W. Marshak, PhD, Neurobiology and Anatomy
Supported by: The University of Texas-Houston Summer Research Program; National Eye Institute, EY06472
Keywords: eye, vision, neuron

The goal of this study was to describe the neural circuit that provides input to parasol ganglion cells, also known as M cells because of their projection to the magnocellular layer of the lateral geniculate nucleus, in the primate retina. An ON parasol ganglion cell was injected with Neurobiotin and serially sectioned for electron microscopy in order to identify its synaptic inputs. Processes presynaptic to the labeled ON parasol ganglion cell primary and higher order dendrites were described, and synaptic connections with other types of neurons were analyzed. Synapses onto dendritic spines were quite rare. One of the processes seen in almost every series is an electron-dense, irregularly shaped amacrine cell process that is invaginated by other amacrine cell processes and synapses with other amacrine cells of the same type. Based on previous experiments using antibodies to choline acetyl transferase, these are likely to be from cholinergic amacrine cells. Several other types of amacrine cells were also presynaptic to the labeled ganglion cell dendrite. Eleven bipolar cell processes presynaptic to the ON parasol ganglion dendrite were analyzed for dyad composition and type of amacrine cell input. Twenty-three dyads were studied of which 4% were ganglion cell/ganglion cell, 52% were ganglion cell/amacrine cell and 43% were amacrine cell/amacrine cell. Of the twelve amacrine cell inputs, 50% were feedback and 50% were feed forward. The majority of the input to this ON parasol ganglion cell was from amacrine cells. The remainder of the input was from bipolar cells, probably the DB5 type.

Orbital frontal lesions in monkeys impairs problem-based learning but not object recognition memory

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Sponsored by: Jocelyn Bachevalier, PhD, Neurobiology and Anatomy
Supported by: The University of Texas-Houston Summer Research Program; MH58846 and HD35471
Keywords: rule-learning, delayed nonmatching-to-sample, spatial memory, novelty preference

Orbital frontal lesions impaired object recognition memory as assessed by delayed nonmatching-to-sample (DNMS) task, but not spatial memory (Meunier et al., 1997). The deficit was accounted by a difficulty in acquiring the rule (linking reward to stimulus novelty) inherent to DNMS. Here we tested whether the deficit could be due to either a difficulty in novelty detection or short-term memory. Rhesus monkeys with orbital frontal cortex lesions and unoperated controls were tested on a new object recognition task not requiring rule learning. In this task, animals were familiarized with a stimulus for 30 sec, and after a 10-sec delay, presented with the familiar and a novel stimulus. Recognition memory was inferred by longer looking times at novel stimuli. For object memory, stimuli were pictures of objects presented over an invariant background. For spatial memory, familiar and novel stimuli consisted of an ensemble of 5 objects, which were either presented in a different configuration (experimental) or differed by one object (control). No significant group differences were found in either condition. The sparing of spatial memory is in agreement with previous findings but not that of object memory. That is, operated monkeys showed normal novelty preference, hence normal ability to detect, process, and remember objects at the same delay in which they failed to learn DNMS. Thus, the orbital frontal cortex does not appear to be critical for perceptual processing or recognition memory per se, but rather mediates problem-based learning.

Evaluation of the role of the amygdala in novelty detection

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Sponsored by: Jocelyne Bachevalier, PhD, Neurobiology and Anatomy
Supported by: The University of Texas Houston-Summer Research Program; MH58846 and HD35471
Keywords: temporal cortical areas, monkeys, recognition memory

Electrophysiological studies in monkeys have indicated that the amygdala contributes to novelty detection (Rolls et al., 1993) and may complement the critical participation that the temporal cortical areas, such as perirhinal and entorhinal cortices, make to this process (Xiang & Brown, 1998). There exists, however, no direct evidence that the amygdala plays a critical and necessary role in novelty detection. To test this idea, rhesus monkeys with amygdala lesions and unoperated controls were tested on an object recognition task, involving novelty detection. In this task, animals were familiarized with a stimulus for 30 sec, and after a 10-sec delay, were presented with the familiar and a novel stimulus. Recognition memory was inferred by longer looking times at novel stimuli. The animals were tested on two conditions in which the stimuli consisted of objects presented onto backgrounds that could be manipulated. In the first condition, the novel and familiar objects were presented over the same background used during the familiarization. In the second condition, objects were presented over a new background, i.e., one that differed from the background used during familiarization. Both groups of animals showed strong preference for looking at the novel objects in the two conditions, demonstrating that damage to the amygdala does not affect novelty detection. These data suggest that the amygdala is not critical for novelty detection and that, in the absence of a functional amygdala, temporal cortical areas are able to support this cognitive process.

The effects of voluntary spatial attention on reflexive and voluntary saccades

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Sponsored by: Anne B. Sereno, PhD, Neurobiology and Anatomy
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, MH63340
Keywords: spatial attention, saccade, antisaccade, reflexive, voluntary, inhibition, facilitation, eye movements, reaction time

Previous research has not clarified the effects of spatial attention on eye movements (EMS). Specifically, it is not clear whether attention facilitates, inhibits, or has no effect on EMS. One potential problem is that this research has not distinguished between voluntary and reflexive EMS. The current task will examine the effects of voluntary attention on both saccade and antisaccade tasks. Fifteen subjects, ages 18–52, were instructed to attend to a fixation point and in separate blocks of trials to either make an EM toward (saccade) or away from (antisaccade) the target that was presented. A cue at fixation preceded the onset of the target in each condition; this cue could be either informative (arrow) or neutral. The arrow was always 100% accurate in informing the subject of the direction of the required EM. A variable delay interval (500 or 1000 msec) between the presentation of the cue and the target was used to discourage anticipations. An infrared eye tracking system (ISCAN RK-426) registered subjects' eye position every 6 msec and a computer recorded subjects' response times and the accuracy of their EMS to the target. We found that reaction time was facilitated by the informative cue in the antisaccade task. In the saccade task, however, even though the informative cue also predicted the direction of the upcoming EM, reaction time was not significantly affected. Hence, voluntary spatial attention facilitates voluntary EMS, but did not significantly affect reflexive EMS.

NEUROLOGY

The inhibitory effects of 15-deoxy-^{12,14}-prostaglandin J_2 (PGJ_2) on phosphorylation of IkBa in the rat brain

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Sponsored by: Jaroslaw Aronowski, PhD, Neurology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: immunohistochemistry staining, PGJ_2, Phospho-IkBa

The expression and activation of iNOS by activated microglia and loss of vascular integrity due to inflammation following intracerebral hemorrhage and ischemia are important in mediating brain damage caused by a stroke. The Nuclear Factor-kB (NF-kB) pathway regulates many pro-inflammatory factors and leads to the expression and activation of iNOS, release of NO by microglia, endothelium, and astroglia. NO is highly toxic to the neurons. Recently, PGJ_2, a cyclopentenone prostaglandin, has been shown to produce an anti-inflammatory effect in the pleural cavity inflammation model in rats. The effects of PGJ_2 in the brain are unknown. It is suggested that PGJ_2 anti-inflammatory effect is due to the disruptions of the NF-kB activation pathway by inhibiting the inhibitor-kB kinase (IkB-K) reducing the phosphorylation of IkB, preventing the activation of NF-kB. The ability of PGJ_2 to reduce phosphorylation of IkB will be used as an index of NF-kB activation. 10 mg of the PGJ_2 will be injected into the cerebral cortex of Long Evans Rats for varying lengths of time. The brains will be removed and frozen in chloroform. 20 mm sections will be used for immuno-histochemistry. The presence or absence of Phospho-IkBa staining in the cerebral cortex at the PGJ_2 injection site will predict if in fact the anti-inflammatory effects of PGJ_2 can be achieved.

NUCLEAR MEDICINE

Evaluation of clinical services provided in the Weatherhead PET Center for oncology-related imaging

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Sponsored by: Bruce J. Barron, MD; Vicki Ephron, RN, Nuclear Medicine
Supported by: The University of Texas-Houston Summer Research Program
Keywords: evaluation of positron emission tomography (PET) imaging

The study involves systematic follow up of patients receiving a PET scan for oncologic diagnosis to determine diagnostic accuracy and find ways to improve patient care through greater utility of these scans. PET scan technology is a relatively new field so improvements are still valuable. Patients referred for PET imaging for the purpose of tumor localization were offered enrollment in this study before their scan or within the year following the scan. Those participating had oncology records requested at 3 months post-PET scan and at 1 year. In addition, all subjects were asked for written feedback about their experience with the PET scan. The 3-month physical exam, laboratory, radiology and progress notes records will be compared to the PET report for confirmation of all areas of abnormal uptake seen on PET. This data, plus an optional clinician feedback form, will also be used to see how the clinician was able to use the information provided by the PET scan and will help the radiologist evaluate diagnostic. The 1-year records will provide confirmation of the 3-month data or demonstrate continuing change.

This is an ongoing study and the results are not yet available.

OBSTETRICS AND GYNECOLOGY

Effects of synthetic retinoid (vitamin A derivative) in ovarian cancer chemoprevention

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Sponsored by: Changping Zou, MD, PhD, Obstetrics and Gynecology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: ovarian cancer, retinoic acid, chemoprevention

Epithelial ovarian cancer has the highest mortality rate of any of the gynecologic cancers with a 5-year survival for Stages III and IV (the most commonly diagnosed stage) of 15–20% despite aggressive treatment. Over 70% of ovarian cancers are diagnosed after the cancer has spread beyond the ovary. Two challenges face gynecological oncologists: inability of early detection and resistance to chemotherapy. Early detection and chemoprevention are the important strategies for prevention of ovarian cancer. Among the various chemical or natural compounds used as chemopreventive agents, retinoids appear to be one of the most promising groups of agents. Some data suggests a protective effect of certain retinoids against ovarian cancer. The purpose of the study is to investigate the effect of 4-hydroxyphene-retinomide (4-HPR), a synthetic retinoid, on ovarian cancer cells growth and p53 gene expression. The ovarian cancer cell line OV-CA433 was used in the study. Effect on the growth of OV-CA433 cell line was evaluated in monolayer culture and the expression of p53 gene was analyzed by western blot. The results indicated that 4-HPR inhibited OV-CA433 cells growth in dose dependent manner and 4-HPR induced the p53 gene expression. Conclusion: 4-HPR is a potent growth inhibitor in ovarian cancer cells. The effect of 4-HPR on p53 gene expression needs more clinical and basic study.

Effects of prostacyclin on the motility of sperm

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Sponsored by: Jaou-Chen Huang, MD, Obstetrics and Gynecology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: spermatozoa, motility, prostacyclin

Prostacyclin (PGI_2) is known to relax smooth muscle of blood vessels and inhibit platelet aggregation. Recent studies on transgenic mice suggest that PGI_2 is critical for embryo implantation and pain sensation. Thus, PGI_2 has other functions outside cardiovascular system.

It is known that caffeine and forskolin enhance sperm motility through increased intercellular cyclic-AMP (cAMP). Molecular cloning of PGI_2 receptor suggests binding of PGI_2 receptor will activate adenylcyclase and increase intracellular cAMP. Furthermore, clinical observation associates low sperm motility with low PGI_2 concentrations in the semen. Recently we found PGI_2 makes up 40%–50% of prostaglandins secreted by epithelial cells of human fallopian tubes. These findings lead us to hypothesize that PGI_2 may regulate sperm motility.

To test this hypothesis, we used a computer-assisted semen analyzer (Integrated Visual Optical System Sperm Analyzer, Hamilton-Thorn Research, Beverly, MA) to determine the effect of iloprost, a PGI_2 analog, on the motility of sperm. For positive control we used 8-bromo cAMP. We determined the percentage of sperms that displayed hyperactive movement and recorded other parameters of sperm movement such as: amplitude of lateral head displacement, curvilinear velocity, straight line velocity, average path velocity, and linearity. If iloprost is found to affect sperm motility, we plan to use radio-labeled iloprost to determine the binding characteristics of prostacyclin receptors on the sperm membrane.

Gender analysis of the Cochrane reviews as related to cardiovascular disease

Carrie A. Karvonen, University of Northern Iowa, Class of 2002

Sponsored by: Shahla Nader, MD, Obstetrics and Gynecology; Cynthia L. Phelps, PhD, Health Informatics; Barbara M. Sanborn, PhD, Biochemistry and Molecular Biology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: women, Cochrane, cardiovascular disease, clinical trials

Cardiovascular disease research throughout the past decade has suggested that women may respond differently to treatment and intervention than men. The Cochrane Database of Systematic Reviews is the most widely recognized authority for evidence-based medicine, and contains meta-analyses of literature addressing a specific question. If the Cochrane is to be an authoritative source of clinical evidence, its content must reflect the current status of healthcare with respect to both genders. With a specific interest in the representation of women in clinical trials, we critically searched Cochrane reviews related to cardiovascular disease to determine if women were included in trials, if the results for men and women were analyzed separately, and if there were gender-discordant outcomes for trials in which a sex-specific analysis was conducted. Thirty Cochrane reviews, included in the Cochrane Heart, Hypertension, and Peripheral Vascular Diseases review groups were used in our analysis. Original articles for trials included in each review were retrieved and data was extracted and pooled. We found that of 299 trials, women comprised approximately 29% of the total studied population. Sixty-six of the 299 trials had a sex-specific analysis. Some trials however, included only men or were not available for our study. Of these 66 trials, 12 discussed results in which there was statistically significant discordance ($p < 0.05$) between male and female data. Because of the possibility of gender differences in effectiveness and response to treatment, the Cochrane Library should incorporate gender analysis in its reviews.

Incidence of adhesions after previous cesarean section

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Sponsored by: Vaseem Ali, MD, Obstetrics and Gynecology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: adhesions, laparoscopy, cesarean section

Complications derived from post-surgical intra-abdominal adhesions pose a significant medical and surgical problem. The purpose of this study is to examine the incidence of adhesions following cesarean sections as it has immense impact in future surgical procedures and symptoms of pain, discomfort, and infertility. In this retrospective cohort study, medical charts of 225 female patients were analyzed and 198 patients were enrolled in the study after using inclusion/ exclusion criteria and evaluated for adhesions, and endometriosis. X_2 test and two sample t-tests were employed to compare the variables between patients with and without cesarean sections. $p < 0.05$ was considered significant. Using laparoscopy, adhesions were observed in the following locations: perihepatic, right and left adnexa, anterior and posterior cul-de-sac, RLQ, previous scar, and abdominal. Cesarean section prior to laparoscopic procedure was noted in 43 of the 198 women studied in this analysis. Overall, there was a significant increase in adhesion formation in patients with a C-section. In patients with C-section, 26/43 or 64.47% had adhesion formation compared to only 27/155 or 17.42% in patients without a C-section. The incidence of pelvic and intra-abdominal adhesions is drastically increased after a cesarean section i.e. 60.4% versus 8.3% in the control population, which is statistically significant. There is no significant increase in the adhesions formations in the perihepatic region or the cul-de-sac. Preventive measures include VBAC, minimal surgical intervention, and barrier agents.

OPHTHALMOLOGY AND VISUAL SCIENCES

Ocular findings in computer vision syndrome

Anisa Gire, University of Houston, Class of 2003
Sponsored by: Richard W. Yee, MD, Ophthalmology and Visual Sciences
Supported by: The University of Texas- Houston Summer Research Program
Keywords: computer vision syndrome, dry-eye syndrome

Every year 10 million Americans visit ocular specialists with Computer Vision Syndrome (CVS)-related eye complaints. CVS is a combination of ocular symptoms including eye strain, headaches, blurred and double vision, dry and irritated eyes, photophobia, and neck and/or back pain. The Video Display Terminal (VDT) is believed to be a threat to health and safety due to radiation emission, repetitive strain injuries, and "technostress," which include eyestrain, headaches, and fatigue. A decrease in blinking rates from 20–25 times per minute to 5–10 times per minute, also contributes to these symptoms and is a significant factor in the dry-eye syndrome. Dry eye can also be caused by exposed ocular surface, environmental factors, contact lens wear, age, gender, and systemic medications. In the study, patients will be randomized into two groups and play a computer game for 40 minutes without computer goggles and then play again for 40 minutes with the goggles on. Patients will complete a questionnaire rating personal eye complaints. The patients will receive a complete vision exam and have their ocular tear film measured using a Tearscope. Patients with CVS symptoms are believed to have vertical wave patterns when their tear-film is viewed with the Tearscope, while patients without CVS symptoms appear to have horizontal wave patterns. The computer goggles enclose much of the extraocular surface surrounding the eyes causing ocular humidity to increase while decreasing direct light and glare to reduce CVS symptoms.

Vitreous glutamate concentration in normal and glaucomatous monkey eyes

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Sponsored by: M. L. J. Crawford, PhD, Ophthalmology and Visual Science
Supported by: The University of Texas-Houston Summer Research Program
Keywords: glaucoma, ganglion cell death, glutamate excitotoxicity

Objective: Our purpose was to test the claim that the excitatory amino acid glutamate exists in higher concentrations in the vitreous of glaucomatous eyes. Such evidence would support the glutamate toxicity model, which proposes that retinal ganglion cell death occurs as a result of glutamate excitotoxicity. **Methods:** Experimental glaucoma was induced in one eye of twenty seven adult macaque (M. mulatta) monkeys using argon laser treatment to the trabecular meshwork, while the untreated eye served as a within animal control. Amino acid profiles were determined by two independent laboratories using high-pressure liquid chromatography (HPLC) for vitreous samples drawn from both eyes at time of sacrifice. Samples were also taken from both eyes of normal monkeys to serve as controls. The relative differences in glutamate concentration within and between experimental and normal eyes were calculated. **Results:** The mean glutamate concentrations were statistically identical in normal and glaucomatous eyes. Both laboratories reported mean glutamate concentrations of approximately five micromoles per liter in glaucomatous eyes and the same in normal eyes. **Conclusions:** The excitatory amino acid glutamate is not found at toxic concentrations in the vitreous body of glaucomatous eyes, failing to reproduce previous findings. However, toxic glutamate concentrations may still exist in the retina within the active region of the characteristic scotoma. Experiments measuring glutamate levels within the retina using a glutamate-sensitive microelectrode may support a modified local glutamate toxicity model.

ORTHOPAEDIC SURGERY

Methods of repair of a transverse fracture of the acetabulum

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Sponsored by: Catherine G. Ambrose, PhD, Biomechanics Research Lab Director
Supported by: The University of Texas-Houston Summer Research Program
Keywords: acetabulum, transverse fracture, fixation, stability

Transverse fractures of the acetabulum are a result of high energy impact and they can be recognized by a horizontal fracture line which splits the hemipelvis into two main fragments. It is extremely important to reduce the fracture site, as unreduced fracture may lead to decrease of the weight bearing area and therefore predispose the joint to post traumatic arthritis. Open reduction and internal fixation with the use of variety of screws and plates are commonly used methods in taking care of transverse acetabular fractures. The purpose of this study is to evaluate and compare three different methods of fixation using the LoneStar spring plate, taking into consideration the length of the surgical procedure and surgical approach. 15 synthetic hemipelves will be used for the study. A transverse acetabular fracture will be created in each specimen, and it will be then fixed using one of three different methods, using screws and plates. Each fixed hemipelvis will be supported in a jig (previously designed and constructed) and placed in a Material Testing Machine (MTS). The purpose of the testing is to observe any displacement of the fragments after fixation. The displacement will be measured with the use of two Linear Variable Differential Transducers (LVDTs). The measured displacement will be plotted versus the load applied across the hip joint. The goal is to achieve the stiffest fixation that will guarantee smallest displacement under load.

OTOLARYNGOLOGY

Early development of auditory temporal processing

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Sponsored by: Lincoln C. Gray, PhD, Otolaryngology
Supported by: The University of Texas-Houston Summer Research Program; March of Dimes, #12-FYOO-0166
Keywords: hearing, auditory, temporal processing, alarm calls, sound

The goal of the project was to test the sensitivity of newborn subjects to artificial and naturalistic sounds. Young humans are often tested with clicks, tones, and noise bursts. Researchers believe that newborn humans are actually more sensitive to sounds than can be demonstrated with these artificial stimuli. In order to test this hypothesis an animal model will be used. The project will test the sensitivity of newborn chickens to their maternal alarm calls. Newborn chickens' sensitivity to alarm calls (naturalistic stimuli) will be compared to their sensitivity to white noise bursts (artificial stimuli). The stimulus consists of three different calibrated alarm calls that are transmitted into a chamber at a sound level that the newborn chickens will be able to hear. Depending on the chickens' responses to the stimuli, data will be compiled on their sensitivity to the sounds. It is hoped that the project will help to better understand the methods for testing the hearing of newborn subjects.

PATHOLOGY

Managing patient diagnostic information using an image database application

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Sponsored by: Z. Hong Zhou, PhD, Pathology and Laboratory Medicine
Supported by: The University of Texas-Houston Summer Research Program
Keywords: pathology image, patient information, image database

Recent advances in computing and bioinformatics have offered an unprecedented opportunity for pathologists to improve patient care by efficient information management. One of the major goals in pathology is the acquisition and management of diagnostic images and patient information so that they can be accessed securely and easily by authorized clinicians, from any where at any time. In order to accomplish this goal, we have chosen ImageWhere, a digital image management system, as a framework on which to implement our pathology specific solutions. The ImageWhere software suite consists of three parts: the ImageWhere archiving database, the QuikCap image capture utility, and the ImagePortal browser interface. It provides solutions to image capturing, archiving, indexing, annotation, exporting, and administration. The hierarchical organization of the ImageWhere was employed in our design to create a catalog, which consists of various forms and folders. Our form contains 10 descriptive fields associated with pathology images. We have also established the QuikCap capability for capturing new images from diagnostic microscopes directly. This allows for images to be easily archived and automatically indexed. These images and their associated annotations can then be shared by multiple clinicians on the Internet through the ImagePortal web interface. Therefore, from image capturing, storing, categorizing, to data retrieving, we have obtained an integrative solution for managing pathology image data.

Acknowledgment: we thank Dr. Robert Hunter and Dr. Andy Nguyen and Jeremy Corry for their advice and technical support throughout this project.

Digitoxin-like immunoreactivity from consumption of Chinese medicine Chan Su

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Sponsored by: Amitava Dasgupta, PhD, Pathology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: Chan Su, digitoxin-like immunoreactivity, FPIA (fluorescence polarization immunoassay), EMIT (enzyme multiplied immunoassay)

Chan Su is a traditional non-prescription Chinese medicine that is prepared from the skin gland of toads. It is widely used for treatment of tonsillitis and sore throat and has recently displayed some cardiotonic potential with effects such as stimulation of myocardial contraction, anti-inflammatory effect, and pain relief.

These possible cardiotonic effects of Chan Su are attributed to its content of bufadienolides such as bufalin, cinobufagin, and resibufogenin. Bufalin is known to block vasodilation and increase vasoconstriction, vascular resistance and blood pressure by inhibiting Na+, K- ATPase. Structural similarity of bufalin to digoxin, a confirmed cardiotonic medicine, further strengthens the case for Chan Su.

To evaluate its efficacy, a study using mice is to be conducted. Mice are fed 75 mg/kg of Chan Su and blood is drawn before, and 1 and 2 hours after feeding. The fluorescence polarization immunoassay (FPIA) for digoxin and the enzyme-multiplied immunoassay (EMIT) for digoxin will be used to detect digoxin-like immunoreactivity within serum. Increased levels of this immunoreactivity after administration of Chan Su will confirm the medicine's usefulness.

Mannose receptor mediated phagocytosis and nitric oxide burst in macrophages

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Sponsored by: Chinnaswamy Jagannath, PhD, Pathology and Laboratory Medicine
Supported by: The University of Texas-Houston Summer Research Program
Keywords: macrophage, nitric oxide, mannose receptor, iron oxide

Macrophages are major components of the immune system of human beings. They have the ability to recognize and phagocytose pathogens using specific receptors, such as mannose receptor (MR), on their surface and kill them using several mechanisms. In the present study, a macrophage cell line was used as model to study these events. Macrophages were incubated with super paramagnetic iron oxide (SPIO) particles with an iron oxide core and a dextran outer coat. They were labeled with a fluorescent probe (FITC) and uptake was monitored by fluorometric measurement of FITC label within the macrophages at an excitation of 485 nm and emission of 530 nm. SPIO was rapidly taken up by the macrophages in two hours, yielding approximately 59 intracellular fluorescence units. SPIO particles were then incubated in the presence of mannan, an inhibitor of MR that is also known to bind to dextran and thus hypothesized to phagocytose SPIO. Mannan caused a 41 % inhibition in the uptake of SPIO confirming uptake through MR. MR mediated phagocytosis leads to free radical synthesis. Macrophages were phagocytosed with SPIO and similar particles coated with a lipid (LPIO) instead of dextran. After 24 hours of incubation, culture supernatants were tested for the presence of nitrite, an indicator of nitric oxide (NO) synthesis by macrophages. Only SPIO was found to induce significant levels of NO, LPIO being negative indicating that only dextran coated SPIO entered through the MR. We suggest that uptake of SPIO through MR was responsible for NO release. Thus, macrophages binding bacterial pathogens through MR probably use NO to kill them.

Nonyl acridine orange decreases adriamycin-induced apoptosis in a quiescent fashion

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Sponsored by: Jeanie B. McMillin, PhD, Pathology and Laboratory Medicine
Supported by: The University of Texas-Houston Summer Research Program
Keywords: apoptosis, adriamycin, non-acridine orange, electron transport

The antineoplastic agent Adriamycin (doxorubicin) is used to treat a wide array of tumors. However, the drug has been shown to increase oxidative stress and cause apoptosis, or programmed cell death, in the heart by binding to the intramitochondrial negatively charged phospholipid cardiolipin and releasing cytochrome c into the cytosol. This cascades further until the cysteine protease Caspase-3 is turned on, leading to cell death. Apoptosis in the heart occurs during ischemia, congestive heart failure, and myocardial infarction. Neonatal rat cardiomyocytes treated with 10 ?M Adriamycin experienced 64 ± 2 fluorescent units/minute/mg protein of caspase-3 like activity after 16 hours of treatment. Cells pretreated with 1?M nonyl acridine orange (NAO) one hour prior to the addition of Adriamycin had a 45% lower amount of caspase-3 like activity (35 ± 1 fluorescent units/minute/mg protein). NAO is believed to slow apoptosis by competing with Adriamycin for a binding site on cardiolipin. Electrophoretic-mobility-shift analysis of nuclear extracts showed decreased binding of cardiac-enriched transcription factor Myocyte Enhancer Factor-2 (MEF-2) and ubiquitous transcription factor Upstream Stimulatory Factor (USF-1) as the concentration of NAO increased. By preventing the release of cytochrome c, NAO not only slows the progression of adriamycin-induced apoptosis, it also shuts off transcriptional machinery in an effort to conserve ATP secondary to the decrease in electron transport. The next challenge would be to create a drug that protects against apoptosis without hindering oxidative phosphorylation in patients taking Adriamycin.

PEDIATRICS

Correlation of anogenital findings and child sexual abuse disclosures

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Sponsored by: Kim K. Cheung, MD, PhD, Pediatrics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: child sexual abuse, anogenital findings, child disclosures

In child sexual abuse cases, medical evidence, victim's age and disclosure all contribute to the prosecution of perpetrators. Studies nationwide report abnormal physical findings concerning for sexual abuse in 10–20% of victims. The purpose of the study is to investigate and correlate anogenital physical findings with disclosure in a regional center where pediatricians/nurse practitioners with expertise in colposcopic evaluation in children examine child sexual abuse victims. At Harris County, Texas, there were approximately 25,000 alleged child maltreatment victims and approximately 3,350 (10%) of these cases concern child sexual abuse in the year 2000. The Children's Assessment Center (CAC) in Houston provides for multidisciplinary evaluation and treatment services for sexually abused children within Harris and neighboring counties. A retrospective chart review was conducted for all children seen at the CAC from June 2000 through June 2001. A total of 1272 charts were reviewed. Preliminary data indicates there are 822 (64.6%) anogenital exams with findings within normal limits, 414 (32.5%) exams with abnormal findings, and 36 (2.8%) incomplete/inconclusive sexual abuse exams. Nonspecific findings, categorized within the abnormal findings, are 123 (9.7%). Nonspecific findings include, but are not limited to, candidiasis, labial adhesions, and genital/anal warts for children under two years of age. The results of our study correspond to the results of similar studies nationwide, establishing abnormal anogenital findings to be relatively uncommon in sexual abuse exams. We plan to conduct further analysis of the data collected in this study to correlate rate of disclosure with physical examination findings.

Awareness of sexually transmitted infections among high-risk adolescents

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Sponsored by: William L. Risser, MD, PhD, Pediatrics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: sexually transmitted infection, adolescents, complications, awareness, symptoms

Purpose: To measure the knowledge of high-risk adolescents about various sexually transmitted infections (STIs), namely their knowledge of the names and symptoms and of facilities that diagnose and treat STIs. **Methods:** At the Harris County Juvenile Detention Center, I administered a confidential, one-on-one interview to females and males 12-18 years old. Questions concerned syphilis, gonorrhea, chlamydia, herpes virus, genital warts, and HIV; and the clinics where teens can receive evaluation and treatment for STIs. **Results:** I interviewed 78 adolescents, 39 females and 49 males, whose mean age was 16 years (range 12–18 years). Of the 39 females, 19 (49%) were African-Americans, 14 (36%) were Hispanics, 4 (10%) were Caucasian/White, and 2 (5%) were Mixed/Biracial. Of the 49 males, 21 (43%) were African-Americans, 17 (35%) were Hispanics, 9 (18%) were Caucasian/White, and 2 (4%) were Mixed/Biracial. Of the 39 females, 61% were able to answer fewer than 50% of the questions concerning their knowledge of the specific sexually transmitted infections, and 15% could answer 75% or more. Of the 49 males, 35% were able to answer fewer than 50% of these questions, whereas 5% could answer 75% or more. In particular, 95% of all subjects were aware that AIDS was not curable but knew almost nothing else about HIV infection. Subjects with a history of STI were more cooperative, knowledgeable, and interested in learning more. Ninety percent of both females and males knew of a clinic or doctor that could provide evaluation and treatment of STIs. **Conclusion:** I found that these female adolescents knew more than males about STIs but that neither sex knew very much. This high-risk group of youth needs much more information.

PSYCHIATRY AND BEHAVIORAL SCIENCES

Mechanism of impulsivity and bipolar disorders

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Sponsored by: Frederick G. Moeller, MD, PhD, Psychiatry and Behavioral Sciences
Supported by: The University of Texas-Houston Summer Research Program
Keywords: bipolar disorders, impulsivity, MHPG

Affective disorders such as impulsivity are often related with certain levels of a particular metabolite in the human body. Specifically, there is a certain metabolite in human blood plasma, 3-Methoxy-4-hydroxyphenylglycol (MHPG), which is believed to account for these impulsivity levels. To further understand the mechanism of this relationship, plasma from the blood of inpatients (with varying cases of affective disorders) in participating centers of the National Institute of Mental Health Clincal Research Branch Collaborative Study on the Psychobiology of Depression, Biological Studies, were extracted, centrifuged, and prepared in 1 ml extracts. These extracts were observed using high pressure liquid chromatography techniques, and the levels of MHPG were measured accordingly. Afterwards, the DNA of the plasma extracts was amplified using PCR techniques, in order to further detect and delineate MHPG levels between extracts. It is hoped that the psychological and clinical meanings of bipolar disorders, in the context of different affective states, can be further understood through continuing research into the same mechanism described earlier.

Neurophysiology of emotion perception in autism

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Sponsored by: Katherine A. Loveland, PhD, CHDR, Psychiatry and Behavioral Sciences
Supported by: The University of Texas-Houston Summer Research Program
Keywords: autism, event related ootential, emotion perception, prefrontal cortex

Autism is a developmental disorder impairing social-emotional behavior. Recent research suggests developmental impairment of the orbitoprefrontal-amygdalar circuit in the brain could underlie these deficits. Many autistic persons rely on cognitive strategies to compensate for socioemotional deficits. Because such strategies are slower than normal emotion processing, rapid presentation of emotional stimuli may directly test the impairment of the orbitoprefrontal-amygdalar circuit and its role in emotional processing. We used Event Related Potentials to examine the neurophysiology of emotion perception in children and adolescents (14 age and IQ matched subjects: 7 autism, 7 controls). Using EEG data from a 128 channel Electrical Geodesics system, subject-averaged ERPs were averaged to produce waveforms, and then statistically analyzed. We recorded ERPs to rapidly-presented pictures in two categories; subjects pressed a key when the infrequent category appeared (visual oddball paradigm: 80% frequent, 20% infrequent pictures). Three conditions included: easy emotions (happy, sad faces), difficult emotions (surprised, frightened faces), and animals (dogs, cats). ERP data were analyzable from 3 Ss with autism and 4 controls, and behavioral data from 5 Ss with autism and 7 controls. 220 ms after stimulus onset (P2a), subjects with autism showed significantly smaller positivity over frontal cortex to emotion conditions but not to animals ($F = 4.47$, $p < 0.05$); they also made significantly more errors in emotion recognition than controls ($F = 5.21$, $p < 0.05$). However, their P2a response to animals was enhanced compared with controls. Results emphasize the importance of prefrontal cortex in evaluating the social significance of stimuli.

Impulsivity in bipolar disorder

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Sponsored by: Alan C. Swann, MD, Psychiatry and Behavioral Sciences
Supported by: The University of Texas-Houston Summer Research Program
Keywords: bipolar disorder, impulsivity, BIS-11, IMT/DMT

It has been proposed that impulsivity is a significant characteristic of bipolar disorder. According to the DSM-IV (1994) criteria, bipolar disorder is characterized by a history of one or more manic, hypomanic, or mixed episodes and generally one or more major depressive episodes. We studied patients previously diagnosed with bipolar I disorder, bipolar II disorder, and secondary bipolar disorder. We measured impulsivity using a self-administered psychological rating scale, the Barratt Impulsiveness Scale (BIS-11), and a Continuous Performance Task, the Immediate and Delayed Memory Task (IMT/DMT). The laboratory measure of impulsiveness is determined by the rate of commission errors, the percentage of responses to numbers that differ from the target response by a single digit. Total BIS-11 scores differed significantly between either manic or non-manic bipolar subjects and matched controls. IMT commission errors, however, were notably elevated only in bipolar subjects in manic episodes; non-manic bipolar subjects were identical to controls. These results suggest that BIS-11 scores measure impulsivity as a stable trait in bipolar disorder, while impulsivity as measured by CPT commission errors is specific to the manic state.

Predicting response to methylphenidate in children with ADHD using fMRI

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Sponsored by: Deborah A. Pearson, PhD, Psychiatry and Behavioral Sciences
Supported by: The University of Texas-Houston Summer Research Program
Keywords: ADHD, fMRI, methylphenidate, psychostimulant

There is currently no objective measure for determining which children with ADHD will respond positively to psychostimulant medication. At present, to determine if a prescribed medication will be effective for a patient, physicians must resort to a trial-and-error method. Dr. Deborah Pearson is currently investigating the use of magnetic resonance imaging (MRI) techniques to predict medication response to the psychostimulant methylphenidate, commercially known as Ritalin. Brain activity is analyzed in scans taken while the patient is on placebo and therapeutic doses. My participation in this study has been primarily to assist in the screening and assessment of patients. Parent and teacher behavioral questionnaires are used to measure behavioral response to medication treatment, along with attention and inhibition tasks, which are used to assess the child's cognitive response to medication. Prior to study entry, the children are given an extensive screening using a variety of psychological and psychoeducational tests. I observed the administration and assisted in the scoring of these measures. From my work on this project, I have learned about not only the symptoms and treatment of ADHD, but also some methods used in psychological research.

RADIOLOGY

Normal gray matter distribution of Tc-99m-ECD in pediatric brain SPECT scans

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Sponsored by: Lamk M. Lamki, MD, FRCPC, Radiology
Supported by: The University of Texas-Houston Summer Research Program
Keywords: Tc-99m-ECD, brain SPECT, region-of-interest (ROI)

Objective: To measure normal relative Tc-99m ethyl cysteinate dimer (ECD) distribution in the gray matter of the brain. **Methods:** Fourteen pediatric patients (ages 7-17) with normal brain SPECT scans were acquired using a 3-headed gamma camera, as indicated by various medical conditions. Tc-99m-ECD distribution was measured by the average number of counts in each region-of-interest (ROI) over 10 regions in transverse sections and 10 in coronal sections of the brain scans. Left-to-right ratios were calculated for each region. **Results:** Regional left-to-right differences varied from 2% to 7%. The left-to-right variations calculated for the parietal and occipital lobes were no more than 2%. These regions had the least variation. The inferior temporal lobe had a right side increase in activity of 7% compared to left. This region had the greatest amount of variation. Regional differences between various regions of cortical gray matter were within 10%. The subcortical gray matter (Basal Ganglia) had about 20% higher activity than the cortical. **Conclusion:** Distribution of Tc-99m-ECD is fairly uniform in normal gray matter of the brain in children. The left-to-right difference of any region is less than 7% in normal children. The region-to-region differences in the cortex are less than 10%. These results agree with results of other brain imaging agents studied previously, such as Tc-99m HMPAO.

SURGERY

Altered cytokine profile in simulated microgravity and its modulation by supplemental nucleotides

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Sponsored by: Anil Kulkarni, PhD, General Surgery
Supported by: The University of Texas-Houston Summer Research Program
Keywords: simulated microgravity, immune system, nutrients, cytokines

Microgravity environment of space has deleterious effects on human physiology. There is immune function decrease in astronauts during space flight and immediate post-flight period and these decreases are in T-cell function and their altered cytokine profile. Cytokines are proteins that are secreted by various leukocytes, to activate other cells, which regulate all-important processes: cell growth, cell activation, inflammation, immunity, tissue repair, fibrosis and morphogenesis. The objective of this work is to investigate the role of nucleotides on cytokines in modulation of immune response under simulated microgravity (SMG) conditions. In this study, we cultured normal lymphocytes in normal gravity of tissue culture flasks and in SMG of rotating wall vessel bioreactor developed by NASA. Cells were cultured with or without PHA and nucleotides and supernatant were quantitated for various cytokines. We used the ELISA Assay Kits to measure all cytokines. In preliminary results, for IFN-g, IL-1 b and IL-2, the samples with PHA and nucleotides had a significantly higher response than the control culture. These results are in agreement with previous *in vivo* experimental results. Additional preliminary results for IL-3 and TNF-a were inconclusive. Further experiments and sampling are needed. We can conclude that supplemental nucleotides can upregulate certain cytokines expression and production in SMG to modulate and improve immune function.

The effects of bacterial toxins on rat ileal mucosal permeability

Paul A. DeLyria, Case Western Reserve University, Class of 2003

Sponsored by: Frank G. Moody, MD, Surgery; Norman W. Weisbrodt, PhD, Integrative Biology and Pharmacology
Supported by: The University of Texas-Houston Summer Research Program; National Institutes of Health, GM38529
Keywords: translocation, fMLP, ileum

Post-traumatic ileus is associated with bacterial overgrowth and the movement, or translocation, of commensal enteric bacteria from the ileal lumen into the circulation and other organs. Translocation of bacteria may be a factor involved in multiple organ failure, but the full mechanism of this potentially fatal process is unknown.

It has been proposed that by increasing gut permeability, more bacteria may be able to translocate. Previous experiments show that formyl-methionyl leucyl phenylalanine (fMLP), a toxin secreted by gram-negative enteric bacteria, has a transient effect on the permeability of the mucosal barrier. We hypothesize that this transient effect will correlate with a change in the concentrations of mucosal pro-inflammatory mediators such as TNF-α.

In order to test this hypothesis, Sprague-Dawley rats were anesthetized with Xylazine/Ketamine. An incision was made down the abdominal midline and both kidneys tied off. Then a 10cm section of the most distal ileum, starting at the ileo-cecal junction, was measured and cannulated at both ends. After 90 minutes of Krebs perfusion, fMLP (10^{-5}M) was introduced and perfusion continued with the new solution for different times up to 1.5 hours. Animals were sacrificed at this point and the concentrations of TNF-α, IL-1ß, IL-6, histamine, serotonin and substance P in the ileal homogenates were determined.

We would expect a correlation between the change in concentration of pro-inflammatory compound and the transient change in permeability observed after fMLP exposure.

DENTAL BRANCH

BASIC SCIENCES

Cardiovascular responses to real life stress

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Sponsored by: Ted D. Pate, PhD, Basic Sciences
Supported by: The University of Texas-Houston Summer Research Program
Keywords: stress, heart rate, reactivity

Stress is an important health risk factor in the life of a dental student. Learning how stress affects the body may help reduce this risk factor in the future. The goal of this study is to determine the effects of stress on the heart rate while a student performs a series of three dental extractions. It is hypothesized that stress will increase the heart rate from baseline levels during the dental extractions, and a decrease in mean heart rate is expected during each sequential extraction due to the rising confidence of the students as they become more familiar with the procedure. To this point, a total of six dental students were monitored by an ECG machine via a wireless transmitter. The heart rates of the students were recorded during a baseline reading and their first and second dental extractions. The mean heart rate increased by 19.9 bpm from baseline levels (73.5 bpm) to the first extraction (93.4 bpm). The results also show a slight decrease in the mean heart rate from the first (93.4 bpm) to the second (91.4 bpm) dental extraction. Based on the results thus far, it appears that the stress of performing a dental extraction increases the heart rate from baseline levels and as the student gains more experience, this response may be attenuated. Continuation of this study should lead to more significant results.

The regulation of osteopontin by oxidative stress

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Sponsored by: Amy L. Ridall, DDS, PhD, Basic Sciences/Prosthodontics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: atherogenesis, osteopontin, oxidative stress, allylamine

Atherosclerosis is a cardiovascular disease characterized by formation of plaques along blood vessel walls. It is believed that these plaques result, in part, from interactions of extracellular matrix components (ECM) and lipids with integrin receptors on the surface of vascular smooth muscle cells (VSMC). Previous in vivo studies demonstrated that subjecting VSMC to oxidative stress produces plaques similar to those found in atherosclerosis, with a characteristic upregulation of the integrin-binding, ECM protein, osteopontin (OPN). To study this upregulation of OPN by allylamine, development and optimization of an in vitro system for cell transfection was required. To accomplish this, time course and dose response experiments were designed to establish the optimal exposure conditions that lead to oxidative stress without cell death. A 4.5K promoter fragment of OPN fused to the luciferase gene was transfected into rat VSMC cells in vitro. Following transfection, cells were treated with allylamine to produce oxidative conditions. Luciferase activity was recorded as a measure of OPN transcriptional activity. Our data demonstrated that the optimal in vitro treatments that best paralleled the in vivo findings was a regiment of treating the cells with 100mM allylamine for 1 hour. A long-term goal of our study is to elucidate the signaling pathways involved in OPN regulation by oxidative stress. To begin to identify these pathways, an experiment to transfect plasmids containing binding sites of common transcription factors into VSMCs, followed by treatment with allylamine, is currently underway.

ORTHODONTICS

Bioconvection and gravitaxis in the calcifying alga *Pleurochrysis carterae*

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Sponsored by: P. Jackie Duke, PhD, Orthodontics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: *Pleurochrysis carterae*, gravitaxis, coccolithophores, space

Bioconvection is the formation of convection patterns within a culture of swimming microorganisms due to their collective behavior. Swimming strains of *Pleurochrysis carterae* exhibit bioconvection when cultured in various containers, affirming the presumed negatively gravitaxic behavior of the cell. These unicells are being grown for preliminary studies relating to an International Space Station experiment that will study the relationship of mineralization and morphogenesis to gravitaxis. In the present study, cells were grown in a completely filled Rose chamber. Wintrack 2000 was used to determine the influence of bioconvection on the direction of cell movement by analysis of videos acquired at different magnifications and cell concentrations. Results show that the cells swim up, concentrating at the top of the container, and then abruptly fall, forming convection currents visualized as thin but dense columns of down flowing cells. The greatest influence on movement of individual cells is seen in or near these columns. Cells detaching from the stream move upward at an angle in part influenced by the convective fluid flows. This angle determines if the cell will reenter the stream, or move into the region between downstreams and swim directly up. Therefore, these cells do not swim independently in a population; however, this effect is diminished at lower densities. The videotaping plan for the experiment aboard the Space Station includes taping with white light and infrared, allowing the question of bioconvection in the absence of gravitaxis and phototaxis to be addressed.

INSTITUTE OF MOLECULAR MEDICINE, BIOLOGY AND BIOCHEMISTRY

RESEARCH CENTER FOR HUMAN GENETICS

Determining the localization of RB15 expression in the mouse model using an EGFP-containing adeno-associated virus

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Sponsored by: Ba-Bie Teng, PhD, Research Center for Human Genetics
Supported by: The University of Texas-Houston Summer Research Program
Keywords: RB15, ribozyme, apolipoprotein B, low density lipoprotein

Elevated levels of low density lipoprotein (LDL) cholesterol and overproduction of apolipoprotein B (apoB), the primary protein component of LDL, are established risk factors for the development of premature coronary artery diseases in humans. RB15, a hammerhead ribozyme, directly reduces the levels of these molecules in hyperlipidemic individuals. Delivered to mice using adenovirus, RB15 binds and cleaves cellular apoB mRNA, and thus decreases levels of functional apoB protein and LDL cholesterol. To further assess the utility of RB15 as a potential hyperlipidemia treatment, our aim is to develop a recombinant adeno-associated virus (rAAV) expressing the reporter gene, EGFP (enhanced green fluorescent protein), in order to determine its target sites in vivo. Through observing the localization of EGFP expression, we can extrapolate this information to the behavior of RB15. To begin this process, PCR-amplified EGFP was ligated into the plasmid AAV, and sequencing was used to confirm the presence of EGFP in the plasmid. EGFP-containing plasmid (10 ug/plate) and pDG helper plasmid (30 ug/plate) were then transfected into 293 cells to produce the recombinant virus (rAAV-EGFP). After transfection, the virus was isolated and purified using an Iodixanol gradient and Heparin Affinity Chromatography. The titer of the rAAV-EGFP, 6×10^8 particles/ul, was determined using real-time PCR. HepG2 cells were then infected with 6×10^9 particles, and concurrently, 9 mice were transduced via jugular, tail, and supraorbital vein injections (6 mice: 6×10^9 particles/animal, 3 mice: 1.8×10^{10} particles/animal). On days 7 and 30 after these injections, mouse organs will be harvested and examined under fluorescent microscope to observe areas of EGFP expression.

RESEARCH CENTER FOR IMMUNOLOGY AND AUTOIMMUNE DISEASES

Gene characterization of mouse carboxypeptidase U

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Sponsored by: Rick A. Wetsel, PhD, Research Center for Immunology and Autoimmune Diseases
Supported by: The University of Texas-Houston Summer Research Program
Keywords: carboxypeptidase U, gene structure, knockout, fibrinolysis

Carboxypeptidase U (CPU) is a plasma zymogen that is a link between coagulation and fibrinolysis. CPU inhibits fibrinolysis by removing carboxyl-terminal lysine and arginine residues from partially degraded fibrin, which decreases the rate of plasminogen activation. Although CPU is activated in vitro by proteins involved in the coagulation and fibrinolytic pathways, there may also be other biological roles for the enzyme. To better understand the role of CPU, the gene was characterized and a knockout construct was designed. A clone containing the CPU full length cDNA was obtained from ATCC and sequenced. A mouse genomic library was screened with a 900 bp probe from the CPU cDNA. Three positive BAC clones were found and DNA was isolated from these clones. The CPU cDNA sequence was also used to search a mouse genomic database, and the CPU gene was found. The gene spans approximately 41 kb and contains 11 exons. The gene structure was used to design a knockout construct that replaces exons 7 and 8 with a neomycin cassette, thereby removing the active site of the enzyme. DNA from the BAC clones was used to PCR a 5' homologous arm that was successfully ligated into a vector containing a neomycin resistance gene. Upon insertion of a 3' homologous arm, the completed construct will be transfected into mouse embryonic stem cells. Genetically modified mice deficient in the CPU enzyme will be created using successfully targeted stem cells. These mice will provide a useful model to study the role of CPU *in vivo*.

M. D. ANDERSON CANCER CENTER

PATHOLOGY

RNA extraction from RNAlater and formalin fixed paraffin embedded uterine tissue

Melissa Muff, University of Northern Iowa, 2002
Sponsored by: Russell Broaddus, MD, PhD, Pathology
Supported by: M. D. Anderson Cancer Center
Keywords: RNAlater, formalin, paraffin embedding, uterus

Following hysterectomy, uterine tumors are fixed in formalin and embedded in paraffin blocks. Hematoxylin and eosin stained slides are cut from these blocks and examined for clinical diagnosis. This traditional process of fixation upholds the morphological properties of the tissue, but does not preserve RNA within tumor cells for further use in research. RNAlater (Ambion), an aqueous solution, preserves cellular RNA within tissues. This project sought to determine the effectiveness of RNAlater tissue fixation in the extraction of RNA from paraffin blocks. Normal and cancerous uterine tissues were collected from patients and either placed in RNAlater and frozen at –20°C or fixed separately in RNAlater or formalin and then embedded in paraffin. RNA extracted from the frozen tissue was used as a positive control. RNA from the RNAlater and formalin paraffin blocks was extracted using Ambion's Paraffin Block RNA Isolation kit. Gels were used to detect total RNA from the samples, and PCR of cDNA was performed to amplify specific sequences. High levels of RNA were consistently extracted from the RNAlater frozen samples. For the paraffin blocks, the RNAlater samples had slightly higher total RNA levels than the formalin samples. However, amplification resulted in comparable bands for both fixatives. Histologically, the cellular detail of the formalin fixed tissue was better than in the RNAlater tissues. Because of inconsistent RNA extraction and inferior histology, the use of RNAlater as a fixative should be restricted to research settings.

ST. LUKES EPISCOPAL HOSPITAL

CENTER FOR ORTHOPAEDIC RESEARCH AND EDUCATION

Ulnar bending stiffness for caucasian men and women at comparable bone mineral densities

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Sponsored by: Gary Kiebzak, PhD, Center for Orthopaedic Research and Education
Supported by: The University of Texas-Houston Summer Research Program
Keywords: mechanical response tissue analysis, bone mineral density, ulna, bending stiffness, body mass index, bone mineral content

Mechanical response tissue analysis (MRTA) is a vibrational technique used to measure ulnar bending stiffness; data are expressed as the product of Young's modulus of elasticity and the cross-sectional moment of inertia (EI) in units of Newton meter squared. The purpose of this study was to compare the EI for men and women with approximately the same bone mineral densities (BMD). Data were collected at Miller Orthopaedic Clinic in Charlotte, NC. BMD (g/cm^2), bone mineral content (BMC, g/cm), and EI measurements for the ulna mid-shaft of the dominant arm were collected in 47 Caucasian women (42 ± 17 yrs) and 23 men (39 ± 12 yrs). The EI for men ($59.6 \pm 20.8\ Nm^2$) was significantly greater than that of women ($32.9 \pm 10.7\ Nm^2$)($P < 0.001$, unpaired t-test). To account for differences in bone size, the following adjustments were made to the EI: EI/BMC: men $28.5 \pm 8.9\ Nm^2/g$ and women $23.2 \pm 5.8\ Nm^2/g$ ($P < 0.001$), EI/BMD: men $72 \pm 20\ Nm^2/(g/cm^2)$ and women $46.5 \pm 11.1\ Nm^2/(g/cm^2)$ ($P < 0.001$), EI/BMI: men $2.3 \pm 0.75\ Nm^2$ and women $1.3 \pm 0.46\ Nm^2$ ($P < 0.001$), EI/ulna width: men $49.9 \pm 12.7\ Nm^2/cm$ and women $31.2 \pm 7.3\ Nm^2/cm$ ($P < 0.001$), EI/weight: men $0.34 \pm 0.11\ Nm^2/lbs$ and women $0.22 \pm 0.07\ Nm^2/lbs$ ($P < 0.001$). These adjustments only partially corrected for differences in bone size between men and women. Differences in material properties between bone tissue of men and women may account for sex differences in EI despite adjustments.

SCHOOL OF HEALTH INFORMATION SCIENCES

HEALTH INFORMATICS

Student degree plan – A web-based application

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The purpose of this project was to transfer the paper form degree plan that students, faculty, and staff are using presently to a web-based application using a database and application server. A web-based application would allow the above users easy access to view and modify the degree plan. In carrying out this project, users were consulted with regards to their usage of the degree plan. The consulted users included Master Degree students of the School of Health Information Sciences, Administration staff, and Dr. Kathy Johnson, a faculty member. Kroenke's Semantic Object Modeling was used to implement users' perception of the student degree plan and to derive the tables used to store data pertaining to the degree plan. The tables were created using MS SQL and are stored in the MS SQL server. Data contained in the tables include the student identification number, course numbers of the courses taken or courses that the student intends to take, semester, year, and credit hours of courses taken, and name of advisors among others. Using SQL, data can be extracted from tables and used to populate the web page. The web page interface was built using HTML and ColdFusion 4.0. ColdFusion is a application development tool that extends HTML capability by providing functions, conditional operators, and database commands. Presently, students are able to view their courses and partial modification to the degree plan is possible.

Research and design of web-based biographies

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The LEARN (Learning Education and Research Network) Project strives to use technology-based teaching in high school curriculums to teach mental health concepts specifically learning and memory. The LEARN curriculum includes hands on activities, web activities, and biographies.

As part of the LEARN team, I designed a biographies web site that profiles scientists whose work focuses on learning and memory. The web site is for high school students, to encourage them to enter scientific fields. In order to get feedback from high school students, a survey was created and given out targeting students (ages 15–21). Results from the survey indicate students use a computer daily, and that they would use a computer to research a career. The students were most interested in salary, longevity, and years of schooling. Questions were then made for scientist interviews of based on the results of the survey.

Next, research was done through the Community of Science and university web sites to find potential scientists to interview. Individuals were chosen based on their research and appeal. The interviews were recorded digitally and footage will be included on the web site.

Additionally, I created the information architecture with the interview data, which includes text, photos, video and audio clips and both a student's and a teacher's version. I also designed an appropriate interface based on Jakob Nielsen's Web Usability. Although the web site is geared towards high school students, it is accessible for all who wish to learn about science careers.

SCHOOL OF NURSING

CENTER FOR NURSING RESEARCH

Age-related differences in female circadian temperature rhythms

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Keywords: circadian temperature rhythms

Objective: Previous research has shown alterations in temperature rhythm in the luteal phase of the menstrual cycle. Altered temperature rhythms are associated with impaired performance. The purpose of the study was to determine whether different phases of the menstrual cycle affect the circadian temperature rhythm. **Methods:** Participants in the time-series design included a normal-cycling 21 year-old woman and one 53 year-old perimenopausal woman. A data logger was worn daily to measure skin temperature every minute. The temperature probe was placed on either the lower outer abdominal quadrant or the breast lower sternal side. The probe was removed only during water activities. The loggers were programmed to measure temperatures between 32°C and 45°C with a resolution of 0.1°C. The individual's activities and menstrual cycles were documented in a daily log. **Results:** Skin temperatures ranged from 33.92 to 37.06, with 53,927 data points for the younger woman and 8,830 for the older woman. Temperature data for the 21 year-old showed a circadian temperature rhythm; the data for the perimenopausal woman showed an ultradian rhythm (21 hours). Analyses determined the best cosine curve fit with the raw data. The parameters were as follows: mesor (rhythm adjusted mean) of 35.49 and 35.51 and amplitude of 0.66 and 0.13 respectively for the two subjects. **Conclusion:** Although the data have not yet been analyzed according to menstrual cycle phases, these findings suggest age differences in temperature rhythms. Future work will need to separate the effects of advancing age from the effects of menstrual cycle phases.

NURSING SYSTEMS AND TECHNOLOGY

Adaptation to extrauterine life

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Keywords: heart rate variability (HRV), neonate

The transition from fetal to neonatal life induces drastic changes in the cardiovascular system of the neonate. When observed, physiologic variability taken during the period of adaptation to extrauterine life provide some insight into how newborns are adjusting to their new environment. However, vital signs alone do not sufficiently predict how well a neonate will progress; similar infants with initially identical vital signs may develop very differently. To examine how these vital signs accurately exhibit infant adaptation, HRV in full term fetuses just prior to birth as well as variability in heart rate, blood pressure, respiration, oxygen saturation, and temperature after birth were observed for both healthy and sick, full and pre-term neonates. Data were also collected on the medications and care the mother and neonate received. The objective of the research is twofold: (1) to better differentiate between the sick baby who needs intervention and the sick baby whose condition is already improving; and (2) to develop a more sensitive tool to identify at an earlier age infants at risk for neurodevelopmental delay. Databases for maternal/fetal and neonatal data organization were also updated.

Due to the damage caused by the flood, results have not yet been fully collected nor analyzed. Meanwhile alternate areas of research were assigned. Light and sound intensity data were collected in a neonatal ICU. A palm-pilot program for nursing home care evaluation was edited. Finally, a web course for an introduction to health informatics class was moved into a more secure programming language and edited.

SCHOOL OF PUBLIC HEALTH

HUMAN GENETICS CENTER

B2AR haplotype SNPs relevance to phenotypic expression in relation to hypertension

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Keywords: B2AR, SNP

Hypertension is a common chronic disease with a complex genetic foundation involving multiple genes. The beta 2 adrenergic receptor (B2AR) is a G-protein coupled receptor that regulates the action of catecholamines in multiple tissues and is encoded by an intronless gene on chromosome 5q31-32 and is known to be associated with hypertension. The B2AR gene has multiple variants in the coding and non-coding regions, including both insertions/deletions and single nucleotide polymorphisms (SNPs) (some altering the encoded amino acid). However, the consequences of these variants and their relevance to particular disorders are largely unknown. Genotyping by sequencing of 50 subjects (25 African-American, 25 Caucasian) revealed 21 polymorphic sites within the region of interest. To determine the haplotype of these individuals the B2AR gene was obtained from genomic DNA by PCR, ligated into pCR 2.1-TOPO vector, transformed into competent TOP10 *E. coli* cells and the plasmids recovered. B2AR gene inserts in the plasmids were then recovered by PCR and sequenced by fluorescence based Sanger sequencing and haplotypes determined. These haplotypes from this population sample will be used to infer the haplotypes from a sample of 1200 African-American/Caucasian subjects in an ongoing case-control study for hypertension status with the objective of revealing any significant statistical correlation between particular haplotypes and hypertensive status.

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Summer Research Program undergraduate participants, 2001