31st ANNUAL MEETING

THE SOUTH CENTRAL CHAPTER OF THE SOCIETY OF TOXICOLOGY

“TOXICOLOGICAL RESEARCH - INSPIRING THE NEXT GENERATION OF TOXICOLOGISTS”

OCTOBER 10-11, 2013

The Department of Environmental Toxicology
Southern University, Baton Rouge, LA

J.K. Haynes School of Nursing Auditorium
THURSDAY, OCTOBER 10
4:00-8:00  Welcome and Registration at the Hilton Garden Inn

5:30-8:00  Networking Reception, Sponsored by Xenometrics, LLC

6:00-7:00  Officer business meeting

6:00-8:00  Toxicology “Trivia Pursuit”

FRIDAY, October 11
8:00-12:00  Registration, J.K. Haynes School of Nursing Auditorium

8:00-8:30  Continental breakfast

Welcome and Keynote Address
8:30-9:00  Welcoming Address

Plenary Session, Sponsored by SOT
9:00-10:00  Keynote speaker
Dr. Steven Safe,
Distinguished Professor of Veterinary Physiology and Pharmacology, Texas A&M University

“The Aryl Hydrocarbon Receptor: A Drug Target?”

Platform Session

10:00-10:15  Platform 1: Y. Wu, US FDA/National Center for Toxicological Research, “Differential Effects of Triclosan on the Activation of Mouse and Human Peroxisome Proliferator-Activated Receptor Alpha.”

10:15-10:30  Platform 2: I.G. Kavouras, University of Arkansas for Medical Sciences, “Epigenetic Alterations Induced by Ambient Particular matter in Raw264.7 Macrophages.”
10:30-10:45  **Platform 3**: N. Mei, US FDA/National Center for Toxicological Research, “In Vitro Mechanism Study of Ginkgo Biloba Leaf Extract-Induced Cenotoxicity.”

10:45-11:00  **Platform 4**: I.R. Mioussse University of Arkansas for Medical Sciences, “Epigenetic Alterations Following Short-Term Exposure To Non-Genotoxic Rodent Hepatocarcinogens.”

11:00-11:15  Coffee break

11:15-11:30  **Platform 5**: M. Carroll-Turpin, LSUHSC-Shreveport, “DAPM Alters Serotonergic Signaling in a Novel Model for Female Specific Pulmonary Arterial Hypertension.”

11:30-11:45  **Platform 6**: A. Alund, University of Arkansas for Medical Sciences, “The Soy-Associated Phytoestrogen, Genistein, Does Not Protect Against Alcohol Induced Osteoporosis in Male Mice.”

11:45-12:00  **Platform 7**: D.R. Sappington, University of Arkansas for Medical Sciences, “Molecular Characterization of Lung Tumors Based on Metabolomic Profiling.”

12:00-12:15  **Platform 8**: B. London, Southern University, “A Cocrystal of M-Chlorbenzoic Acid with Furosemide: Prospective Applications.”

12:15-12:30  **Platform 9**: V.P. Dadhania, University of Louisiana at Monroe, “Inhibition of Secreted Phospholipase A2 (sPLA2) Enzyme Leads to Survival of Lethally Acetaminophen Overdosed Mice.”

12:30-1:00  Break for lunch

**“K-12- Toxicologist” Luncheon  sponsored by Chevron Oronite Company, LLC**

1:00-1:30  Welcome high and middle school student, Chancellor Llorens

1:30-2:00  “Road to becoming a Toxicologist”, Dr. Kenneth E. McMartin

2:00-2:30  Student discussion of project and career

**Poster Session, sponsored by VWR**

2:00-2:15  Break and Poster Set-Up

2:15-3:00  Posters (Odd numbered posters attended)

3:00-3:45  Posters (Even numbered posters attended)

**Award Ceremony, sponsored by Charles River**

4:00-4:30  Award presentations

Adjournment
The South Central Regional Chapter of the Society of Toxicology would like to express its appreciation to the following organization for the support of its 2013 Annual Fall Meeting:

- The Society of Toxicology for providing funding to support keynote speakers and student travel
- Charles River for sponsoring the student awards and Award Ceremony
- Chevron Oronite Company, LLC for sponsoring the “K-12- Toxicologist” Luncheon
- VWR International, LLC for sponsoring the student poster presentation
- Xenometrics, LLC, Stillwell, KS for sponsoring the Thursday Night Network reception
- SUBR’s Timbuktu Academy and LS-LAMP program for supporting Southern University student registration
Officers of the South Central Chapter of the Society of Toxicology
June 1, 2013 to May 31, 2014

**Dr. Wesley Gray, President**
Southern University and A&M College, Baton Rouge, LA

**Dr. Yunfeng Zhao, Vice President**
Louisiana State University Health Sciences Center, Shreveport, LA

**Dr. Martin J. Ronis, Vice President-Elect**
University of Arkansas for Medical Sciences, Little Rock, AR

**Dr. Baitang Ning, Treasurer**
National Center for Toxicological Research, Jefferson, AR

**Dr. Lei Guo, Secretary**
National Center for Toxicological Research, Jefferson, Arkansas

**Dr. Barbara Parsons, Past-President**
US FDA-NCTR, Little Rock, AR

**Dr. Igor Koturbash, Councilor**
University of Arkansas for Medical Sciences, Little Rock, AR

**Dr. Kelly Mercer, Councilor**
University of Arkansas for Medical Sciences, Little Rock, AR

**Dr. Si Chen, Post-doctoral Representative**
National Center for Toxicological Research, Jefferson, AR

**Mr. Arif Yurdagul Jr., Graduate Student Representative**
Louisiana State University Health Sciences Center, Shreveport, LA

2013 SCC-SOT Annual Fall Meeting Local Organizing Committee

Ms. Pamela Brue Johnson, Southern University Baton Rouge, LA
Ms. Deidre Hardy-Street, Southern University Baton Rouge, LA
Dr. Kinesha Harris, Chemistry Department Southern University Baton Rouge, LA
Dr. Caroline Telles, Biology Department Southern University Baton Rouge, LA
Mr. Oval Phillip, Department of Environmental Toxicology Southern University Baton Rouge, LA
Ms. Dacase Falodum, Department of Environmental Toxicology Southern University
Dr. Igor Koturbash, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Dr. Kelly Mercer, Arkansas Children's Nutrition Center, Little Rock, Arkansas
Dr. Yunfeng Zhao, Louisiana State University Health Sciences Center, Shreveport, Louisiana
Dr. Lei Guo, National Center for Toxicological Research, Jefferson, Arkansas
Dr. Martin J. Ronis, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Dr. Baitang Ning, National Center for Toxicological Research, Jefferson, Arkansas
Dr. Barbara Parsons, Past President US FDA-NCTR, Little Rock, Arkansas
South Central Chapter of the Society of Toxicology
2013 Annual Meeting
“Toxicological Research - Inspiring the Next Generation of Toxicologists”

**K-12 Toxicologist Luncheon**

1:00 – 2:30 p.m., Friday, October 11, 2013
Smith Brown Memorial Union Cotillion Ballroom
Southern University and A&M College

**Program**

Greetings and Introductions .................................................. Dr. Caroline Telles
Assistant Professor of Biological Sciences

Welcome .............................................................. Dr. James Llorens
Chancellor, Southern University and A&M College

Welcome .............................................................. Dr. Robert H. Miller, Jr.
Interim Dean, College of Sciences and Agriculture

*Lunch is served*

Introduction of Speaker .................................................. Dr. Heather Kleiner
American College of Education

“Road to Becoming a Toxicologist”
Dr. Kenneth E. McMartin
Professor, Department of Pharmacology, Toxicology and Neuroscience
Louisiana State University Health Sciences Center (LSUHSC) at Shreveport

Acknowledgement of K-12 Visitors ........................................ Dr. Kinesha Harris
Assistant Professor of Chemistry

Introduction of Students and Remarks ......................... K-12 Teachers and Students

Remarks .............................................................. Oliver Cyprian
Chevron Oronite Company, LLC

Remarks .............................................................. Patricia Sikes
Charles River Labs

Closing Remarks ............................................................ Dr. Wesley Gray
President, SCC-SOT
Professor of Environmental Toxicology
Greetings,

As Interim Dean of the College of Sciences and Agriculture at Southern University at Baton Rouge (SUBR), I am honored and privileged to welcome you to the 2013 Meeting of the South Central Chapter of the Society of Toxicology (SCC-SOT). Indeed, we are most honored that SCC-SOT chose SUBR as the host site for its 2013 meeting.

Over the past 31 years, SCC-SOT has fostered scientific teaching, learning, and research among toxicologists in academia, government, and industry. While concentrating your efforts in the states of Arkansas, Louisiana, Mississippi, Oklahoma, and western Tennessee, your impact in the area of toxicology is felt nationwide and ultimately worldwide; for persons trained in toxicology in the regions of your concentration have gone on to contribute to teaching, learning, and research nationwide. And any nationwide knowledge is always destined to become worldwide.

Scientific knowledge needed to solve toxicological problems is critical to the health and well being of a society. The importance of making toxicology awareness a part of our culture cannot be overstated. Accordingly, I applaud you for your program of getting kids involved in thinking toxicologically at an early age. Only by way of such programs can we continue to advance teaching, learning, and research in the all-important field of toxicology.

At SUBR we are currently undergoing academic reorganization. As a part of this reorganization, we have brought biology, chemistry, and toxicology together to form the Department of Biological Sciences, Chemistry, and Environmental Toxicology. In so doing, we are acknowledging our recognition of the fact that toxicology is an interdisciplinary area whose problems require interdisciplinary expertise to solve. Our environmental toxicology faculty at SUBR has always consisted of chemistry and biology professors as well as toxicology professors. So in formally bringing all three disciplines together under one umbrella, we are simply formalizing an academic environment that fosters a more realistic approach to tackling scientific problems. In keeping with goals of SCC-SOT, future summer high school research interns in chemistry, biology, and toxicology at SUBR will be working on research problems that promote a holistic learning experience in science in which toxicology comes into play.

Again it is my honor and privilege to welcome you. May your 2013 Annual Meeting at SUBR be a most productive one.

Sincerely,
Dr. Stephen H. Safe is Distinguished Professor of Veterinary Physiology & Pharmacology and Director of the Center for Environmental and Genetic Medicine Institute of Biosciences and Technology at Texas A&M University Health Science Center. He received his D.Phil in Bioorganic Chemistry from Oxford University in Great Britain in 1965 and postdoctoral fellowship in biochemistry at Harvard University.

Dr. Safe research focused on the molecular biology of hormone/growth factor-induced gene expression in breast cancer cells, the development of new mechanism-based drugs for treatment of breast and other cancers and the differential activation of estrogen receptor a (ERa) and ERb by endocrine disruptors. One of his current research area focuses on the aryl hydrocarbon receptor (AhR), a nuclear helix-loop-helix transcription factor which forms a ligand-induced nuclear heterodimer with the AhR nuclear translocator (Arnt) protein. Research in this laboratory is focused on the molecular mechanism of crosstalk between the AhR and estrogen receptor (ER) signaling pathways in which the AhR inhibits estrogen-induced gene expression. The antiestrogenic activities of some AhR agonists are also being developed as drugs for clinical treatment of breast and endometrial cancers in women. Research on estrogen-dependent gene expression in various cancer cell lines is focused on analysis of several gene promoters to determine the mechanisms of ERa and ERb action. This includes several genes that are activated through interactions of the ER with Sp1 protein and other DNA-bound transcription factors.

Dr. Safe has been recognized with many award and honors, including: Sigma Xi Award for Excellence in Research (1976), Queen's Silver Jubilee Medal in 1978; Distinguished Achievement Award in Research in 1984 from Texas A&M University; 1989-1994 - Burroughs Wellcome Toxicology Scholar Award from 1989-1994; Sid Kyle Chair in Toxicology in 1991 and 2007 he was awarded the Distinguished Lifetime Toxicology Scholar Award by Society of Toxicology. Dr. Safe has authored or coauthored over 700 pre review paper trained numerous graduate students and postdoctoral fellows, and serves as reviewer for NIH.
K-12 Toxicologist Luncheon Speaker

Kenneth E. McMartin, Ph.D.

“The Long And Winding Road To Becoming A Toxicologist”

Kenneth E. McMartin is professor of Pharmacology, Toxicology & Neuroscience at Louisiana State University Health Sciences Center, Shreveport, LA. Dr. McMartin received his Ph.D. in Pharmacology from The University of Iowa in 1977 and Postdoctoral Fellow training at The Karolinska Institute in Huddinge, Sweden, from 1977 – 1979. He is currently Vice President Clinical and Translational Specialty Section for The Society of Toxicology and have served on several elected committees including been officer of South Central Chapter of The society of Toxicology and The Society of Toxicology Education Task force. Dr. McMartin received numerous accolades including Kenneth Morgareidge Award in Toxicology, American Academy of Clinical Toxicology fellow and Society of Toxicology Translational Impact Award. Dr. McMartin has been an educator for over thirty years and has trained five master level students, seven PhD levels student and over six postdoctoral fellows. Dr. McMartin research interest focuses mechanisms of toxicity of alcohols and glycols, renal toxicology, regulation of folate metabolism, tissue culture, folate transport. His current research focuses on determining the mechanism by which a substance is toxic and utilizing this information to develop improved therapies for poisonings. His laboratory discovered and developed the drug fomepizole, which is now the standard-of-care antidote used in treating methanol and ethylene glycol poisonings.
Southern University’s Award in Environmental Toxicology and Environmental Science

The “T.M. Tate Award for Outstanding Abstract or Oral Presentation in Environmental Toxicology/Environmental Science” by an undergraduate student and the " R.H. Miller Award for Outstanding Abstract or Oral Presentation in Environmental Toxicology/Environmental Science” by a graduate student is been established by The College of Sciences and Agriculture at Southern University to honor the contributions of Dr. Robert H. Miller Jr. and Dr. Twintillia M. Tate. The first Awards will be presented to students at the Society of Toxicology South Central Chapter Annual Meeting that will be held in Baton Rouge, October 10-11, 2013. Two undergraduates’ awards ($150 and $100) and two graduate awards ($150 and $100) will be awarded to Student Researchers at the meeting.

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In memory of

"T.M. Tate Award for Outstanding Abstract or Oral Presentation in Environmental Toxicology/Environmental Science"

Twintillia M. Tate, PhD., Professor of Biological Sciences and Environmental Toxicology, Southern University Baton Rouge from 1991 to 2004. Dr. Tate received her BS, Cum Laude, in Biological Sciences from Southern University at Baton Rouge in 1970 and went on to earn her M.S. degree in 1972. She obtained her PhD. Degree in Toxicology in 1989 from at Louisiana State University in Baton Rouge, LA and started her academic career as an Assistant Professor of Biology at Southern University Baton Rouge, LA in 1991.

During her 29 years of service as a professor of biological sciences at Southern University, Dr. Tate was a trailblazer among educators. She made contributions to society and academia by training students at the undergraduate and graduate levels, publishing thirty research articles, receiving numerous research and training grants, and serving on various academic and public boards. She was an absolutely dedicated teacher and a super-mentor for both graduate and undergraduate students at Southern.

Dr. Tate’s services to the university went well beyond the class room. She served as a Councilor for South Central Chapter of Society of Toxicology, (2002-2003), President of National Institute of Science, (2001-2002), Faculty mentor and advisor for Louisiana Alliance for Minority Participation (LAMP), Phi Sigma Honor Society at Southern University; Beta Kappa Chi Scientific Honor Society; and for Sigma Xi Honor Society In addition, she was a member of several local, regional and national scientific organizations that included The American Microscopical Society; The American Society of Parasitologists; The Association of Women in Science, and the Louisiana Society of Microscopy.

Dr. Tate will always be remembered for her love of community and her promotion of academic excellence.
**Regulatory Science Awards**

Three Regulatory Science Awards will be given to the best research presented at the Society of Toxicology South Central Chapter Annual Meeting that will be held in Baton Rouge, October 10-11, 2013. Two awards will be given to Student Researchers ($50 and $150 value) and one to Postdoctoral Researcher ($200).

These awards are sponsored by the Regulatory Science Program at University of Arkansas for Medical (Little Rock, AR). The UAMS Graduate Certificate in Regulatory Sciences provides an extension to the Ph.D. student’s existing toxicology/pharmacology training. The Certificate in Regulatory Sciences focus provides post-doctoral fellows or graduate students a unique component to their training that sets them apart from other classically trained scientists when seeking employment opportunities, whether they seek jobs in governmental regulatory agencies, regulated industries, or academia. The Certificate in Regulatory Sciences serves both full-time and part-time students interested in expanding their knowledge of regulatory science. The training provides a more competitive background for regulatory science-based careers. The need for increased training in Regulatory Sciences is highlighted in a recent Institute of Medicine (IOM) report entitled, “Strengthening a Workforce for Innovative Regulatory Science in Therapeutics Development: Workshop Summary” (2011).

A primary goal of the UAMS program is to provide students with insights into the complexities of the laws, regulations, policies, risk assessments, risk-benefit analyses and risk management processes. This training provides graduates with a working knowledge of regulatory science and provides leaders in regulatory science for industry, government, and academia. To learn more about the UAMS Regulatory Sciences certificate program, go to [http://publichealth.uams.edu/academics/certificates/certificate-in-regulatory-science/](http://publichealth.uams.edu/academics/certificates/certificate-in-regulatory-science/).
DIFFERENTIAL EFFECTS OF TRICLOSAN ON THE ACTIVATION OF MOUSE AND HUMAN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA

*Yuanfeng Wu¹, Qiangen Wu¹, Frederick A. Beland¹, Mugimane G. Manjanatha², and Jia-Long Fang¹
¹Division of Biochemical Toxicology, ²Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA.

Triclosan is a broad spectrum anti-bacterial agent widely used in many personal care products, household items, medical devices, and clinical settings. Induction of liver tumor by triclosan has been reported in mice following long-term oral exposure, and is proposed to be associated with the activation of peroxisome proliferator-activated receptor alpha (PPARα). The present study examined the effects of triclosan on PPARα activation and the downstream events in mouse hepatoma Hepa1c1c7 cells and human hepatoma HepG2 cells. The concentrations of triclosan inhibiting 50% cell growth were similar for both cell lines. In the mouse PPARα reporter assay, triclosan increased PPARα activity with an EC₅₀ value at 7.3 µM. In contrast, triclosan acted as an antagonist of human PPARα by suppressing both the basal and the potent PPARα agonist GW590735-induced luciferase activity. The transcriptional expressions of PPARα and its target gene acyl-coenzyme A oxidase 1 (ACOX1) were elevated in Hepa1c1c7 cells treated with triclosan for 4 h, but not in HepG2 cells. Western blotting analysis showed that triclosan increased the protein level of ACOX1 in Hepa1c1c7 cells but decreased its level in HepG2 cells. DNA synthesis and apoptosis, two key events related to PPARα activation mediated hepatocarcinogenesis in rodents, were further determined using BrdU incorporation assay and caspase 3/7 activity assay, respectively. Treatment of Hepa1c1c7 cells with triclosan enhanced DNA synthesis and suppressed transforming growth factor beta-mediated apoptosis. In contrast, opposite or no effects were observed in HepG2 cells. These data demonstrated that triclosan had similar cytotoxicity upon mouse Hepa1c1c7 cells and human hepatoma cells but had differential effects on the activation of PPARα, the expression of its target ACOX1, and the downstream events DNA synthesis and apoptosis between the mouse and human hepatoma cell lines.
EPGENETIC ALTERATIONS INDUCED BY AMBIENT PARTICULATE MATTER IN RAW264.7 MACROPHAGES

*Ilia G. Kavouras¹, Isabelle R. Miouss¹, Marie-Cécile G. Chalbot¹, Nukhet Aykin-Burns², Xiaoying Wang³, Alexei Basnakian³, Igor Koturbash¹

1- Department of Environmental and Occupational Health, 2- Division of Radiation Health, 3- Department of Pharmacology and Toxicology, UAMS, Little Rock, Arkansas

Respiratory mortality and morbidity has been associated with exposure to particulate matter (PM). *In vitro* experiments suggest involvement of cytotoxicity, oxidative stress, and inflammation in development of PM-associated pathological states. In the current study, we analyzed the short-term epigenetic response to PM exposure in the RAW264.7 macrophage cell line. PM was collected using a high volume sampler in Little Rock, AR. Analysis revealed that PM was composed mainly of Al and Fe, and also contained Cu, Zn, Pb, V, As, Ni and Cr. The water soluble organic fraction was dominated by aliphatic and carbohydrate fragments and minor quantities of aromatic components. Exposure of RAW264.7 cells to various concentrations of PM (0 – 600 μg/ml) showed signs of cytotoxicity, inflammation and oxidative stress, and compromised the cellular epigenome at non-cytotoxic concentrations of PM. The latter was evident by alterations in the methylation and expression of repetitive elements-associated DNA (LINE1, SINE B1, SINE B2, major and minor satellites), as well as expression of genes, involved in maintenance of normal patterns of DNA methylation and expression of repetitive elements. These results suggest that epigenetic mechanisms might contribute to the pathogenesis of PM-associated respiratory diseases.
IN VITRO MECHANISM STUDY OF GINKGO BILOBA LEAF EXTRACT-INDUCED GENOTOXICITY

Nan Mei¹, Haixia Lin¹, Xiaqing Guo¹, Stacey L. Dial¹, Lei Guo², Mugimane G. Manjanatha¹, Martha M. Moore¹
¹Division of Genetic and Molecular Toxicology, ²Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Ginkgo biloba has been used for many thousand years as a traditional herbal remedy and its extract has been consumed for many decades as a dietary supplement. In the current study, the in vitro genotoxicity of ginkgo biloba leaf extract and its eight constituents were evaluated using the mouse lymphoma assay and the Comet assay, and the underlying mechanisms of ginkgo biloba leaf extract-associated genotoxicity were explored. Ginkgo biloba leaf extract, quercetin, and kaempferol resulted in a dose-dependent increase in the mutant frequency and DNA double-strand breaks (DSBs). Western blot analysis confirmed that both quercetin and kaempferol activated DNA damage signaling pathway with increased expressions of γ-H2AX and phosphorylated Chk2 and Chk1. In addition, ginkgo biloba leaf extract produced reactive oxygen species and decreased glutathione levels in L5178Y cells. Loss of heterozygosity analysis indicated that ginkgo biloba leaf extract, quercetin, and kaempferol treatments resulted in extensive chromosomal damage. These results suggest that ginkgo biloba leaf extract and its two constituents, quercetin and kaempferol, are mutagenic to the L5178Y cells with induction of DSBs. Quercetin and kaempferol may be responsible for ginkgo biloba leaf extract-induced genotoxicity.
Platform #4

EPIGENETIC ALTERATIONS FOLLOWING SHORT-TERM EXPOSURE TO NON-GENOTOXIC RODENT HEPATOCARCINOGENS

*I.R. Miousse¹, L.A. Murphy², M.R. Schisler², M.J. LeBaron², R.J. Rasoulpour², and I. Koturbash¹.
¹University of Arkansas for Medical Sciences, Little Rock, AR, ²Dow, Midland, MI.

The current product safety assessment paradigm identifies adverse apical effects to establish a no-observed adverse effect level (NOAEL). Hepatocellular carcinogens induce apical liver effects via genotoxic and non-genotoxic (epigenetic) modes of action. However, identification of epigenetic effects is not routinely included in product safety assessment. In the current study, following seven day exposures to the rodent non-genotoxic hepatocarcinogens, Phenobarbital (PB) and Clofibrate (CF), we evaluated both apical and epigenetic endpoints in the livers of male F344 rats to determine if these effects occur in a similar dose response/temporal manner. Exposure to PB (0; 5, 25, and 100 mg/kg/day) or CF (10, 50, 250 mg/kg/day) resulted in dose-dependent increases in relative liver weights and transcript levels of Cyp2b1(PB) and Cyp4a1(CF) along with hepatocellular hypertrophy and hepatocellular proliferation. Pyrosequencing-based analysis of LINE1 repetitive element revealed a significant decrease in liver DNA methylation after treatment with 100 mg/kg/day PB while the expression of LINE1 was unchanged. There were no epigenetic changes observed in kidney, indicating compound and tissue specificity of epigenetic alterations. Altogether, these findings suggest that epigenetic parameters, such as methylation of LINE1, occur within the same dose response as apical endpoints and may serve as early and sensitive biomarkers of exposure to carcinogenic doses of non-genotoxic compounds. Funded in part by the UAMS CTCA UL1TR000039 and KL2TR000063 (IK), ABI (IK), and Dow (RR).
DAPM ALTERS SEROTONERGIC SIGNALING IN A NOVEL MODEL FOR FEMALE-SPECIFIC PULMONARY ARTERIAL HYPERTENSION

*M. Carroll-Turpin, Valeria Hebert, Heather Wensler, Tanya Chotibut, Tammy Dugas. Dept. of Pharmacology, LSUHSC-Shreveport, LA

Pulmonary Arterial Hypertension (PAH) is a cardiovascular disorder characterized by elevated pulmonary artery pressure as a result of arterial wall thickening. Patients survive, on average, 2.8 yr after diagnosis and are 3-4 times more likely to be women than men. The few available therapies only slow progression. Our purpose was to develop a relevant animal model of PAH in order to identify sex differences that contribute to disease progression, and in doing so, identify potential new treatment strategies. 4,4′-Methylenedianiline (DAPM) is an aromatic amine used industrially in the synthesis of polyurethanes. Chronic, intermittent treatment of rats with DAPM results in medial hyperplasia of pulmonary arterioles, exclusively in females, coupled with increases in pulmonary arterial pressures. After 12 wk, significant increases in plasma levels of endothelin-1 (ET-1) and serotonin (5-HT), but decreases in nitrite (NO₂⁻), were observed in females but not males treated with DAPM. In females, ET-1 and 5-HT were significantly correlated with peak pressure gradient, an indirect measure of pulmonary arterial pressure. DAPM-treated females also exhibited increased levels of the serotonin transport protein 5-HTT in their pulmonary arteries. 5-HT is known to induce HPVSMC proliferation and its role in PAH has been established. In culture, DAPM stimulated human pulmonary artery smooth muscle cell (HPVSMC) to take up 5-HT and to proliferate. Human pulmonary artery endothelial cells (HPAEC) were stimulated to release 5-HT. Ongoing studies are aimed at addressing the mechanism(s) of action by which DAPM induces these sex-dependent effects.
THE SOY-ASSOCIATED PHYTOESTROGEN, GENISTEIN, DOES NOT PROTECT AGAINST ALCOHOL INDUCED OSTEOPOROSIS IN MALE MICE

*A.W. Alund,1,2, C. Yang,1, K.E. Mercer,1,2, L.J. Suva2 T.M. Badger,1,2 and M.J. Ronis1,2
1Arkansas Children’s Nutrition Center* and the 2University of Arkansas for Medical Sciences, Little Rock, AR, USA.

Chronic alcohol consumption leads to increased risk of osteoporosis by increasing osteoclast activity and decreasing osteoblast activity in bone. While these effects can be reversed by estradiol, it is also suggested that soy diets can exhibit protective effects against bone loss in men and women because they contain phytoestrogens such as genistein (GEN). To study the effects of genistein, male mice received an EtOH diet, or an EtOH diet with genistein (250 mg/kg) for 8 weeks. Ex vivo microCT analyses of formalin-fixed tibias showed a significant decrease in bone morphology in the EtOH and EtOH+GEN groups compared to the pair-fed (PF) and PF+GEN controls. However, there was an increase in trabecular thickness in the PF+GEN group compared to the PF P<0.05, and increased osteoblastogenesis as measured by alkaline phosphatase (ALP) staining in bone marrow cultures taken from PF+GEN and EtOH+GEN treated femurs compared to EtOH alone, P<0.05. Analysis of the femur RNA showed significant increases in markers of both osteoblastogenesis and osteoclastogenesis. In cell culture, GEN alone was able to significantly increase the number of ALP-stained colonies, but unlike estradiol treatment, genistein also simultaneously increased the RANKL:OPG ratio, P<0.05. These findings show that GEN does not protect against alcohol-induced bone loss because of its dual role in increasing overall all bone turnover. Supported in part by R01 AA18282 and the Carl L. Nelson Chair in Orthopedic Creativity, UAMS.
Lung cancer is the leading cause of cancer-related deaths in US. For the majority of patients (70%), the extent of the disease precludes complete surgical resection and treatment relies solely on radiation, chemotherapy or a combination of both. Unfortunately, these treatments improve survival only minimally and are accompanied by considerable adverse side effects. Despite immense research efforts over the last 20 years, the mean survival of lung cancer patients has only increased by 13 days. This relatively poor median survival is attributed to inadequate therapy selection. Current therapy selection is mainly based on the histopathologic examination of needle biopsies obtained during bronchoscopy. There is growing concern that these classifications and stratification are insufficient to predict treatment response of individual tumors. To complement current histopathological-based tumor classification, we investigated the suitability of metabolomics to improve tumor classification. Therefore, metabolomic profiles were obtained from various lung cancer cell-lines. Data were analyzed for molecular features characteristic for cell-lines derived from adenocarcinoma (AdenoCa) or squamous cell carcinoma (SqCCa). Data analyses revealed features that are capable of clearly separating AdenoCa, SqCCa, and lung fibroblasts from each other. Subsequently, the studies were extended to specimen from 30 lung cancer patients. Similar to the cell-line experiments, metabolomic features were obtained and analyzed for characteristic features of patients with AdenoCa, SqCCa, or non-malignant specimen. The analysis of the human specimen revealed unique features that are suitable biomarkers for tumor classification. Our data suggest that metabolomic profiling is a promising approach to tumor classification and to distinguishing biopsies from lung cancer patients with AdenoCa versus patients with SqCCa.
A COCRYSTAL OF M-CHLOROBENZOIC ACID WITH FUROSEMIDE: PROSPECTIVE APPLICATIONS

*B. London,1 M. Claville,2 F. Fronczek,3 and R.M. Uppu.1* 1 Environmental Toxicology PhD Program, Southern University and A&M College, Baton Rouge, LA; 2 School of Science, University of Hampton, Hampton, VA; 3 Department of Chemistry, Louisiana State University, Baton Rouge, LA

Furosemide is a highly used diuretic for the treatment of hypertension and edema and, to a lesser extent, hypercalcemia. This furan containing compound is of interest due to its toxicity which is not well understood. The free furan itself is a known hepatocarcinogen and toxicant as studied in rats and mice. The epoxide metabolite of furans, formed in CYP450-mediated oxidations, can isomerize to highly reactive electrophilic intermediates such as cis-2-butene-1,4-dial. We have performed the oxidation of furosemide with m-chloroperbenzoic acid (m-CPBA), and isolated various epoxide and isomerized products in support of our efforts to understand this type of toxicity mechanism, and to also identify potential biomarkers for furosemide in humans. During the separation and drying of the products of the furosemide-m-CPBA reaction, we observed the formation of crystals in the mother liquor (the organic layer). Analysis of these crystals by X-ray crystallography revealed a nonahydrate cocrystal of furosemide (starting material) with that of m-chlorobenzoic acid (an inadvertent contaminant or the reduced product of m-CPBA). Analogous to the known properties of cocrystals of furosemide with nicotinamide and their pharmaceutical importance, we believe that the cocrystals of furosemide with m-chlorobenzoic acid could have useful applications in drug development and may lead to formulations with improved potency, solubility, and stability. Therefore, this serendipitous discovery may be an important application for improving furosemide bioavailability. [Support: NSF HRD-1043316 ACE Implementation grant; *correspondence: rao_uppu@subr.edu.]
Platform #9

INHIBITION OF SECRETED PHOSPHOLIPASE A2 (sPLA2) ENZYME LEADS TO SURVIVAL OF LETHALLY ACETAMINOPHEN OVERDOSED MICE

*VP Dadhania¹, JR Latendresse² and HM Mehendale¹. ¹Department of Toxicology, University of Louisiana at Monroe, Monroe, LA, USA; ²Toxicologic Pathology Associates, National Centre for Toxicological Research, Jefferson, AR.

Present study was designed to test whether inhibiting secreted phospholipase A₂ (sPLA₂), a death protein, with a specific inhibitor, BPPA [5-(4-benzyloxyphenyl)-4S-(7-phenylheptanoylamino) pentanoic acid], prevents the expansion of liver injury initiated by a lethal overdose of acetaminophen (APAP) in mice. Male Swiss Webster mice (25-30g) were treated with a lethal dose of APAP (600 mg/kg, ip, in warm 0.45% NaCl) followed by a single dose of either BPPA (20 mg/kg, ip, in DMSO 3 ml/kg) or DMSO vehicle (3 ml/kg, ip) alone injected at 2, 4 or 8h after APAP administration. Survival and mortality were recorded over the next 14 days. Plasma alanine aminotransferase (ALT) and sPLA₂ activities were measured in the mice on alternate days from days 1 to 13. ALT and sPLA₂ activities increased sharply in the mice treated with APAP either alone or followed by DMSO (3 ml/kg, ip) led to 80% mortality. In contrast, mice treated with BPPA at 2 and 4h after the APAP administration exhibited similar rise in sPLA₂ and ALT activities on day 1 declining thereafter and suffered only 10 and 30% mortality, respectively. Covalent binding of ¹⁴C-APAP-derived reactive metabolite to liver protein, hepatic glutathione (GSH) depletion, and hepatic GSH:GSSG ratio did not change after the APAP overdose in the mice receiving the BPPA intervention. Ninety, 70, and 60 percent of the mice treated with BPPA at 2, 4, and 8h after the lethal dose of APAP, respectively, survived. We conclude that timely intervention by the administration of the sPLA₂ inhibitor, BPPA, prevented the expansion of APAP-induced liver injury and death of the mice after the lethal challenge with APAP.
REGULATION OF NADC-1 AND NADC-3 TRANSPORTERS BY DIGLYCOLIC ACID IN HUMAN PROXIMAL TUBULE CELLS IN VITRO

*C. Jamison, G.M. Landry, C.L. Dunning, K.E. McMartin. Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University-Health Sciences Center, Shreveport, LA

Diethylene glycol (DEG), a chemical found in common household products, has caused mass poisonings over the years in various countries, with acute renal failure as the typical sign of exposure. Diglycolic acid (DGA) has been discovered to be the main toxic metabolite of DEG; DGA is taken up in rat kidney cells and produces toxicity in human proximal tubule (HPT) cells. The probable transport system of DGA into kidney cells is a sodium-dependent dicarboxylate transporter (NaDC-1) found on the apical side of the proximal tubule cells; the basolateral bound NaDC-3 is suggested to be a lesser-involved co-transporter. The purpose of this study was to determine whether low dose DGA alters the regulation of NaDC-1 or NaDC-3 in HPT cells. It was hypothesized that DGA would upregulate the NaDC-1 transporter and to a lesser extent, NaDC-3. Human proximal tubule cells were grown to confluency in flasks and treated over a 48 hour period with either growth media at pH 7.4 (control), growth media at pH 6, growth media at pH 7.4 with either the addition of 1 mM DGA or 1 mM citrate. Replacement of fresh media to corresponding flasks was done at 24 hours. Cell lysates were processed using polyacrylamide gel electrophoresis (SDS-PAGE) with antibodies specific for NaDC-1 or NaDC-3 transporter protein at 65kD or 67kD, respectively. Bands were normalized to actin at 42kD. Preliminary data shows a down-regulation of NaDC-1 with 1mM DGA as compared to pH 7.4 and pH 6 controls. NaDC-3 does not appear to be affected by low doses of DGA. These studies indicate minimal alterations in regulation of the transporters due to exposure to substrates (DGA and citrate). Following the determination of the transport system that DGA uses in its mechanism of intracellular accumulation and toxicity, more efficient treatment options can be designed to target the uptake process of DGA.
Poster #2

**BENZO[**A]**PYRENE-MEDIATED CHANGES IN LIVER GENE EXPRESSION AND EGG DEPOSITION FOLLOWING A DIETARY EXPOSURE IN ZEBRAFISH**

*T. Tillman 1, C. Thornton2, J. Corrales2, and K.L. Willett2*

1Department of Chemistry, Tougaloo College, Jackson, MS

2Pharmacology and Environmental Toxicology Research Program, University of Mississippi, University, MS.

Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (BaP), are ubiquitous environmental contaminants derived from incomplete combustion of carbon. PAHs are of concern because they are toxic to aquatic life and are suspected human carcinogens. This study evaluated whether the CYP1 and GST mRNAs were induced by BaP in zebrafish livers following a 22 day dietary exposure. Adult zebrafish (2 females x 2 males, N=10 tanks per treatment) were fed 2% body weight/day flake food treated with 0, 10.5, 114, 1013 µg BaP/g flake (equivalent to 0, 0.21, 23, and 20 µg BaP/g fish/day). BaP was not detected in embryos (~8 hpf) spawned from exposed parents despite good extraction recoveries. Liver gene expression of CYP1A, CYP1B1, CYP1C1, and GSTπ was quantitated using qRT/RT-PCR (n=5). Constitutive expression of GSTπ and CYP1A mRNA was higher compared to CYP1C1 and CYP1B1. Although not statistically different, there was higher CYP1B1, CYP1C1, and GSTπ mRNA expression in BaP-treated females compared to controls (78-, 13-, and 11-fold induction, respectively). In exposed males, CYP1A mRNA expression was dose-dependently induced while in females it was not. Our results show that hepatic CYP1B1, CYP1C1, and GSTπ expression was increased by dietary BaP, and these genes should be further investigated for their role in carcinogen bio-activation. Supported by NIEHS R21ES019940.
UP TAKE, INTERNALIZATION AND QUANTIFICATION OF LHRH TAGGED GOLD COATED SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES IN CANCEROUS MCF-7 CELLS

S. Khiste,1 S. Batra,2 S. Jeyaseelan,2 C.S. Kumar,3 and R.M. Uppu.1,* 1 Environmental Toxicology, Southern University, Baton Rouge, LA; 2 School of Veterinary Medicine, LSU, Baton Rouge, LA; 3 CAMD, Louisiana State University, Baton Rouge, LA.

Previous studies from our laboratory have shown that gold coated superpara-magnetic iron oxide nanoparticles (SPIONs@Au) linked to LHRH can selectively kill cancer cells expressing receptors for LHRH. In these studies, we used cysteamine to cross link SPIONs@Au to the carboxy terminal of LHRH. In the present study, we synthesized SPIONs@Au linked to LHRH through the amino terminal using 12-mecaptododecanoic acid (MDDA) as spacer. The resulting conjugates, SPIONs@Au-MDDA-LHRH were studied for their uptake and internalization by LHRH receptor over expressing MCF-7 cancer cells. When incubated SPIONs@Au-MDDA-LHRH (0.2 mg/mL) for 24 h, we found substantial uptake (180 pg/mL; measured by ICP) of the nanoparticles by MCF-7 cells. TEM studies show evidence of clumps of SPIONs@Au-MDDA-LHRH indicating their uptake. The nanoparticulate clumps were found located inside the membranous compartment together with cell organelles like mitochondria and ended up in late endosomes (autophagosomes), confirming the endocytic pathway of uptake. Further studies are underway to examine the selective tumorocidal activities of SPIONs@Au-MDDA-LHRH under the influence of low strength magnetic field. These studies would pave way for designing nanomaterials with tumorocidal properties and better management of cancer patients. [Support: NSF HRD-1043316 ACE Implementation grant; *correspondence: rao_uppu@subr.edu]
Poster #4

IDENTIFICATION OF HOXA1 DIRECT TARGET GENES BY CHIP-CHIP

*Dominique Townsend, Xiaoping Yi, and Eduardo Martinez-Ceballos. Health Research Center, Department of Environmental Toxicology, Southern University A&M College, Louisiana

Hoxa1 is a transcription factor known to regulate embryonic patterning, development, cell proliferation and/or differentiation. The human HOXA1 protein has also been shown to act as an oncogene in various cell types. Hoxa-1 encodes proteins that bind specifically to a DNA sequence and regulate the expression of DNA. However, the direct gene interaction of Hoxa-1 has not been well characterized due to a lack of known target genes. Using a tagged Hoxa1 expression construct, we identified putative Hoxa1 direct target genes by performing global chromatin immunoprecipitation (ChIP)-chip assays. Our results were compared to published Hoxa1-related cDNA microarray reports as a first step on the validation of our ChIP-chip findings. Here, we analyze protein interactions among gene pairs and their functional relationships to determine strong Hoxa1 gene associations. We investigated Hoxa1 gene interactions using pathway analyses, and validated replicated commonalities amongst research data sets. Our identification of direct Hoxa1 target genes will enable further studies of the consequences of aberrant expression of Hoxa1 during embryonic development and/or cellular transformation (Support: NIGMS/IDeA grant number P20GM103424).
**Poster #5**

**MATRIX COMPOSITION MODULATES OXIDIZED LDL-INDUCED INFLAMMATION**

*A. Yurdagul Jr.*, A.W. Orr

LSUHSC-Shreveport: Cell Biology and Anatomy¹, Department of Pathology²

Endothelial cell activation during early atherogenesis is regulated by blood flow patterns and the accumulation of oxidized LDL (oxLDL). Since matrix composition regulates flow-induced endothelial cell activation, we sought to examine whether matrix composition similarly affects oxLDL-induced endothelial cell activation. Fibronectin matrix deposition occurs early during atherogenesis, and endothelial cells on a fibronectin matrix show enhanced oxLDL-induced proinflammatory gene expression (ICAM-1, VCAM-1) and monocyte binding. In contrast, adhesion to basement membrane proteins limits oxLDL-induced inflammation. OxLDL treatment activates the fibronectin binding integrin alpha 5 beta 1, and preventing signaling through alpha 5 beta 1 inhibits oxLDL-induced NF-kB activity and VCAM-1 expression. Furthermore, inhibiting alpha 5 integrin signaling in atherosclerosis prone mice reduced plaque size and macrophage content. These data suggest that the transition from basement membrane proteins to a transitional fibronectin-rich matrix enhances the inflammatory response elicited by oxLDL.
Poster #6

DEVELOPING THE INTERACTIVE CHROMATIN MODELING PROGRAM

J. Bell1, R. Abcede2, W. Johnston3, J. Liman4, V. Bamburg5, and T. C. Bishop3
1Department of Computer Science, Southern University at Shreveport-Bossier
2Wossman High School, Monroe City
3Departments of Physics and Chemistry, 4Biomedical Engineering, and
5Chemical Engineering at Louisiana Tech University

The Interactive Chromatin Model web server (ICM-Web, www.latech.edu/~bishop) is a tool to model our present understanding of how chromatin folds experimentally. ICM-Web integrates both bioinformatics and computational biology allowing the user to input a sequence of DNA and choose from several different energy models and nucleosome placements. The ICM program operates using El Hassan's algorithm. The current version of ICM-Web evolved rapidly from its original design. The rapid evolution produced an unstructured mix of FORTRAN 77 and FORTRAN 90 and the primary limitation is that the program allows only one type of nucleosome footprint. It was decided in moving forward with this program that ICM is to be redesigned either using C++ or a later version of FORTRAN. The hypothesis is that since both FORTRAN and C++ are both high-level languages that there will be no noticeable difference in the processing time. To compare the two programs, a segment of the ICM code was translated into C++. Then both the FORTRAN and C++ segments were ran processing DNA sequences ranging from 10 base-pairs to 1 million. The DNA sequences were identical and each set of data was ran three times to ensure accuracy and the test were all run on the same machine. The results were that starting at base-pair of 100 C++ ran 42% faster. When base-pair of 1 million was ran C++ ran 51% faster. We can conclude that moving forward that C++ would be a better for programming because it has a much faster processing speed.
Poster #7

STRUCTURAL DYNAMICS OF THE S4-S5 LINKER IN THE ACTIVATION OF VOLTAGE-GATED SODIUM CHANNELS

*Annie P. Clark¹, V. Sree Chalamalasetti², and Sudha Chakrapani²

¹Department of Chemistry, Southern University Baton Rouge, LA 70813
²Department of Physiology and Biophysics, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106

Eukaryotic voltage-gated sodium channels (Naᵥ) are large transmembrane proteins that regulate the electrical conduction system of the heart. Naᵥ dysfunctions are associated with disturbances underlying cardiac arrhythmias. Understanding the relationships between the structure and functions of Naᵥ will further enhance therapeutic treatment for cardiac dysfunctions. NaChBac, a voltage-gated sodium channel discovered in the halophilic bacterium (Bacillus halodurans), is a homotetramer of separate 6-TM which shows strong homology to Naᵥ making it a good model to study voltage-gated sodium channel. The goal of this project is to understand protein motions associated with channel activation, with particular emphasis on the S4-S5 linker which connects the voltage sensor to the channel pore. Upon membrane depolarization, the S4-S5 linker couples outward S4 movement to the opening of the channel pore. The study involved NaChBac protein expression, purification, site-directed spin-labeling, membrane reconstitution, and electron paramagnetic resonance spectroscopy (EPR). Presently, we found that the S4-S5 linker in the activated channel takes up a conformation at the lipid-water interface. This study set the grown work for understanding the activation mechanism of Naᵥ voltage-gated sodium channel.
SYNTHESIS OF AZIDE LIGANDS FOR PAMAM DENDRIMERS

*M. County, L. Luce, J. Manono, S. DiMaggio, and B. Hester

Southern University at Shreveport-Bossier, Xavier University of Louisiana, Chemistry Department, New Orleans, Louisiana 70125, USA

In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world. Current treatments of cancer include invasive and destructive techniques such as radiation and surgery, with hidden and toxic side effects. One way of combating the side effects of surgery and chemotherapy is by using targeted drug delivery made from Poly (amido amine) (PAMAM) dendrimers. Our task was to synthesis azide Ligands for the conjugation of PAMAM dendrimers. Dendrimers are starbranch polymers that can be synthesized by generation, increasing in size and number of terminal amines. Small functional molecules, such as cell targeting agents, drugs, and dyes, can attach to the terminal amines creating a multifunctional macromolecule. Unfortunately, these conjugation reactions result in a distribution of products varying in numbers of conjugates which are difficult to separate from each other. The azide ligand is very polar and allows prep-HPLC separation of the populations. It will also give a unique point of attachment for the small functional molecules to control the number of conjugates per polymer. We believe by controlling the stoichiometry of conjugates that this will aid nano-delivery systems transition by eliminating polydispersity.
**Poster #9**

**TARGETED DELIVERY OF AD-32 (VALRUBICIN) TO OVARIAN CANCER AND BREAST PRE-MALIGNANT LINES VIA RHDL NANOPARTICLES**

*A.R. Harrison*, R. Johnson, N. Sabnis PhD, A.G. Lacko PhD.

\(^1\)Department of Biological Sciences, Southern University, Baton Rouge, LA. \(^2\)Department of Molecular Biology and Immunology, UNT Health Science Center, Fort Worth, TX.

Targeted drug delivery methods have been very promising and prominent in cancer research. Scavenger receptor class B member 1 (SR-B1) receptors facilitate the cellular uptake of cholesteryl esters from high density lipoproteins (HDLs). This property of HDLs makes it an attractive target for drug delivery. **The purpose** of this study was to compare the cytotoxic effect of rHDL-AD-32 and free AD-32 in high SR-B1 expressing ovarian cancer cells (SKOV-3) and pre-malignant breast cells (MCF-10A). **Methods:** SKOV-3 cancer cells and MCF-10A cells were treated with free AD-32 and rHDL-AD-32 nanoparticles at increasing concentrations for 24 hours. The expression levels of LDL and SR-B1 were determined using Western Blot analysis. **Results:** SKOV-3 and MCF-10A cells showed an increased inhibitory effect in the presence of rHDL-AD-32 nanoparticles as compared to free AD-32. Free AD-32 treated SKOV-3 and MCF-10A cells in serum-free media exhibited an IC\(_{50}\) at concentrations of 21.5uM and 7.5uM, respectively. **Conclusion:** Under these conditions, nanoparticles containing AD-32 were more effective than the free AD-32. (Support: Collaborative Undergraduate HBCU-STP Grant PC094536 from DOD-PCRP to Dr. Jamboor K. Vishwanatha and a La-LEQSF-EPS(2013)-SURE-98 award to A.R. Harrison.)
UPF1 PROTECTS FORELIMB FUNCTION IN AN ANIMAL MODEL OF ALS

*K. L. Jackson, R.D. Dayton, and R.L. Klein. Department of Pharmacology, Toxicology, and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA 71130.

Amyotrophic lateral sclerosis (ALS) is the most common degenerative disease of motor neurons. ALS is characterized by a rapid and progressive paralysis that ultimately causes death. In post-mortem ALS samples, the neuropathology is commonly characterized by abnormal aggregations of transactive response DNA binding protein, 43 kDa (TDP-43). We use a viral vector (adeno-associated virus) encoding TDP-43 to induce paralysis of the hindlimbs and forelimbs. In this study, we used the model to determine therapeutic efficacy of an empirically derived target gene. In a yeast model of TDP-43 overexpression, up-frameshift protein 1 (UPF1) protected against cytotoxicity (Ju et al., unpublished). TDP-43 and UPF1 were co-expressed to determine if UPF1 could improve motor function. Controls included TDP-43/Empty vector, UPF1 alone, Empty vector alone, and untreated subjects. While the TDP-43/Empty rats displayed both hind- and forelimb paralysis, notably, the TDP-43/UPF1 rats retained forelimb motor function. The UPF1 treatment also improved rearing behavior (p<0.05) and performance on a rotarod at early but not late stages (p<0.05). There are limited treatment options for ALS, and preservation of forelimb function would be an improvement in quality of life. These positive results support the long-term goal of targeting UPF1’s protective mechanism with a gene or small molecule therapy.
Thin film composite (TFC) membranes, having an extremely thin but effective polymer film as the separating layer, are the leading materials for screening the tiniest solutes from water. In order to drive down energy costs for operating such membrane plants, thinner and more efficient membranes are needed. Current commercial membranes for removing salt from seawater are made via interfacial polymerization, where monomer A (a diamine dissolved in water) reacts instantaneously with monomer B (a triacid chloride dissolved in an organic solvent) at the interface between the two immiscible fluids. These monomers react to form a highly crosslinked, dense aromatic polyamide film that can separate and filter out 99+% of dissolved ions from water. In this project, we instead use a layer-by-layer approach to fabricate the separating layer, where monomer A and monomer B are sequentially and repeated reacted on a surface to form the ‘active’ layer. We created ‘active’ layers made from the reaction of trimesoyl chloride (monomer A) with a variety of diaminos and a triamine (monomer B), in an effort to explore the role of monomer structure on the ability of the membrane to remove salt from water. (Support: Science and Engineering Alliance (SEA) & NSF Grant 70NANB13H)
DIFFUSION OF FUNCTIONALIZED DENDRIMERS INTO MOUSE ES CELL AGGREGATES

*Niharika Mente, *Humberto Munoz, and *Eduardo Martinez-Ceballos

*Department of Biology, Chemistry, and Environmental Toxicology, #Department of Physics and Mathematics, Southern University and A&M College, Louisiana

The controlled release and delivery of drugs with target specificity is the main aim of pharmaceutics involved with drug delivery. A safe and targeted delivery is essential to improve the efficacy and efficiency of a particular drug as it minimizes the undesired side effects. Nano biotechnology has helped foster the development of nano-scale pharmaceutical delivery of the drugs and has been employed for the directed differentiation of stem cells along specific cell lineages. Liposomes, cyclodextrins, dendrimers, etc., have been extensively used in recent times as drug delivery models due to their ability to alter the physiochemical, kinetic, body distribution, delivery properties of hydrophobic drugs. Dendrimers are of special interest since they possess different surface functionalities and the ability to encapsulate a wide group of guest molecules. We were particularly interested in studying the diffusion of Generation 5 (G5) dendrimers into mouse Embryonic Stem (mES) cell aggregates known as Embryoid Bodies or EBs. For this, we treated EBs with chromophore-functionalized dendrimers and examined their diffusion by confocal microscopy. We observed that dendrimers could readily penetrate various EB cell layers in as short as 24 hours after application. In some cases, labeled dendrimers could be detected in the cell nucleus, which supports their use for the delivery of ligands for nuclear receptors. Further discussion on the diffusion properties of functionalized dendrimers into EBs is presented here. Our studies may have implications on areas such as ES Cell Differentiation and Regenerative Medicine.
A COMPARISON OF VITAMIN D DEFICIENCY IN ADULT PATIENTS

*C. Newton¹, M. T. McFarland², MD and B. Hester³ Ph.D.

¹Southern University at Shreveport-Bossier, ²Department of Multicultural Affairs, Louisiana State University in Shreveport, LA

This study is designed to establish patterns or factors which affect the incidence of Vitamin D Deficiency as well as to compare levels of Vitamin D Deficiency in adult patients. The data was analyzed to determine patterns of Vitamin D deficiency by age groups and gender. Patients were selected from a data bank of a local primary care clinic. Researchers studied demographic information including age, race and sex of patients as well as levels of Vitamin D in the body as possible factors in Vitamin D metabolism. Results of these findings were divided into three categories based upon severity of the Vitamin D deficiency in order to obtain an adequate amount of information in to possibly develop a correlation between the different cases of Vitamin D Deficiencies. Through the course of the experiment, there was a noticeable pattern between the different levels of deficiency. There was a noticeable relationship between genders and age. There was an area of the age groups that had the highest occurrences as well as a gender having the same result as well. The women in the two age groups 50-59 and 60-69 had the highest occurrences of Vitamin D Deficiencies. While the men had a high level of occurrences in the age groups of 50-59 and 70-79. The most astonishing factor was that the age group 50-59 had slightly more than half of the total number of patients used for the overall analysis.
INTERACTION BETWEEN NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS AND HIV-1 PROTEINS IN PROMOTING HIV-ASSOCIATED ENDOTHELIAL DYSFUNCTION

*K. Nichols, M. Glover, S. Xue, and T. Dugas. Department of Pharmacology, Toxicology, and Neuroscience, LSU Health Sciences Center, Shreveport, LA

HIV patients on long-term nucleoside reverse transcriptase inhibitors (NRTI) treatment are at a higher risk for cardiovascular disease. We hypothesize that NRTI increase mitochondrial reactive oxygen species (ROS) and promote endothelial dysfunction, leading to atherosclerosis. Moreover, HIV-1 proteins themselves have been shown to induce vascular ROS production through the activation of NADPH oxidase (NOX). Thus, we further hypothesize that HIV-1 proteins may synergize with NRTI to dramatically exacerbate the onset of endothelial dysfunction. To first test whether mitochondrial ROS have a causal role in NRTI-induced endothelial dysfunction, we utilized mice overexpressing manganese superoxide dismutase (MnSOD), a mitochondrial antioxidant enzyme. MnSOD transgenic and wildtype mice were treated with either zidovudine (AZT), lamividine (3TC), or vehicle in the drinking water for 6-8 weeks and endothelium-dependent vasorelaxation was measured in isolated aortic rings. MnSOD overexpression attenuated both AZT- and 3TC-induced endothelial dysfunction. To examine the interaction between NRTI and HIV-1 proteins, Tg26 and wildtype mice were treated with either AZT or vehicle in the drinking water for 6-8 weeks and vessel reactivity was measured in aortic rings. Note that Tg26 mice express a gag/pol-deleted HIV-1 transgene; thus, they exhibit viral protein expression but are not infectious. HIV-1 overexpression did not alter endothelium-dependent vasorelaxation in either strain. However, AZT reduced endothelium-dependent vasorelaxation in both the Tg26 and the wild-type mice. Ongoing in vitro experiments are being conducted to further determine the possible synergistic relationship between NRTI and HIV-1 proteins. Nevertheless, mitochondrial and extra-mitochondrial ROS production appears to have a causal role in HIV-associated endothelial dysfunction.
Poster #15

UV RADIATIONS ON EARTH

*Uchenna Mbadozie, and Lawrence Henry. Center of Research in Physical Sciences Topic. College of Science, SUBR University LA (Support: National Aeronautics and Space Administration)

Ultraviolet Radiation are electromagnetic waves which are part of the electromagnetic spectrum and have wavelengths between X-rays and visible light. Their Spectrum is divided as follows: Vacuum UV (wavelengths: 40-190 nm), Far UV (wavelengths: 190-220 nm), UV-C (wavelengths: 220-290 nm), UV-B (wavelengths: 290-320nm), and UV-A (wavelengths: 320-400 nm). The sun is the primary natural source of UV radiation. The ozone layer absorbs UVC rays preventing them from reaching the earth. Most of the UV radiation on earth are UV-A’s. It is important to know that the longer the wavelengths, the less harmful it is to the human body.

The purpose of our research is to obtain a profile of the UV radiation intensity with altitude above the earth. To accomplish this, an experiment was carried by a balloon 100,000ft above the earth surface. Our results showed that the intensity of UVA is greater than UVB and there were almost no UVC intensity. This is consistent with previously accepted result.
Poster #16

CHLAMYDIA MAJOR OUTER MEMBRANE PROTEIN (MOMP) INDUCES HIGH IFN-γ AND T-CELL PROLIFERATIVE RESPONSES IN MICE

*A. Pearson¹, S. Dixit², S. R Singh³ V. Dennis⁴ and B. Hester⁵
¹Southern University at Shreveport-Bossier, ²Center for Nano Biotechnology and Life Sciences Research Alabama State University

Chlamydia trachomatis genital infection is a worldwide public health problem. Considerable effort has been expended on developing an efficacious vaccine. The murine model of C. trachomatis genital infection has been extremely useful for identification of protective immune responses and in vaccine development. Although a number of immunogenic antigens have been assessed for their ability to induce protection, the majority of studies have utilized the whole organism and its major outer membrane protein (MOMP) as vaccine candidates. To begin to identify the immune-stimulatory regions with T-cell epitopes, we immunized three groups of mice at two-week interval as follows: (i) Group1 (PBS + incomplete Freunds adjuvant (ii) Group 2 (live C. trachomatis) (iii) Group 3 (rMOMP + incomplete Freunds adjuvant). Mice were sacrificed two weeks after the last immunization, and purified T-cells isolated from spleens of immunized mice were restimulated in vitro with Concanavalin A, UV-inactivated C. trachomatis and rMOMP. T-cell samples from mice were analyzed by cytokine ELISA for IFN-γ production and the MTT assay for T-cell proliferation. Our results revealed that rMOMP–stimulated T-cells induced maximum production of IFN-γ.
INTRACELLULAR UPTAKE OF SUCCINATE AND DIGLYCOLIC ACID INTO HUMAN KIDNEY PROXIMAL TUBULE CELLS VIA SODIUM DICARBOXYLATE TRANSPORTERS

*C.N. Robinson, G.M. Landry, C.L. Dunning, K.E. McMartin. Department of Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center, Shreveport, LA

Diglycolic acid (DGA) is one of two primary metabolites of diethylene glycol (DEG), hypothesized to be toxic and in many cases, fatal. DGA is a dicarboxylic acid, with a structure similar to the TCA cycle intermediate, succinate. It has been suggested that DGA and succinate are taken up by the same Na⁺-dicarboxylate transporter (NADC) located in the proximal tubule cells of the kidney. This study compared the intracellular uptake of DGA and succinate, via apical and basolateral NADC transporters. Human kidney proximal tubule cells were cultured until confluent, then subcultured into 24 well plates containing membrane inserts to measure apical and basolateral uptake. Using radiolabeled ¹⁴C substrates, uptake was measured at increasing time points and concentrations for both succinate and DGA, along with measurements of sodium dependence of the NADC transporter. Succinate results showed that cellular uptake from the apical and basolateral membrane increased with increasing concentrations, with a higher level of basolateral uptake of DGA, compared to apical uptake, over time. The uptake of DGA also appeared to increase with increasing concentrations, both apically and basolaterally, with more uptake from the basolateral membrane and with only the basolateral transporter showing evidence of sodium dependence. These results suggest that DGA and succinate transport have differing characteristics in human kidney cells.
A stroke, also known as a “brain attack”, is a type of cardiovascular disease (CVD) that occurs when the brain is unable to get the blood it needs. While stroke most often occurs in older individuals, anyone can have a stroke at any time and at any age. There are two types of stroke: ischemic and hemorrhagic. Ischemic strokes are rare in individuals from 18 to 45 years of age, but can occur, with recent estimates of 4.9% of all strokes in the United States. Strokes can cause negative long-term physical and mental effects, most of which can be even more detrimental if presented during young adulthood. Young adults do not generally consider themselves at risk of having a stroke. However, many risk factors that can potentially lead to onset of stroke are common among college-aged individuals, including hypertension, obesity, migraine, diabetes, and smoking. Additionally, cardiovascular risk factors can present in young adulthood, increasing stroke risk. A recent population-based research study determined that substance abuse significantly increases the chance of stroke, and regular use of nicotine, cocaine, and alcohol have been proven to be associated with both ischemic and hemorrhagic stroke. Other drugs, such as amphetamines, have been associated with hemorrhagic stroke events. The purpose of this survey research was to assess the level of stroke awareness among college-aged students (aged 18 to 30).
Acesulfame potassium (ACK) is an artificial sweetener present in over 4,000 food products and is marketed under the trade names Sunnet and Sweet One. It is about 200 times sweeter than sucrose, but like saccharine, has a bitter aftertaste. Due to its high thermal stability, ACK is often blended with other sweeteners to give a more sugar-like taste and mask aftertaste and possibly camouflaging for better consumer acceptance. The daily intake of ACK is around 1g by a healthy individual on a Western diet and is claimed to be transparent to metabolism and is excreted as is. Although an FDA-approved sweetener, the long term toxicological effects of ACK are not well understood. ACK is present in waste water effluent (>100 ng/L) and thus is likely to seep into the ground water and cause health effects. Therefore, in the present investigation, a method of ozone-mediated destruction of ACK was undertaken. In comparison with indigo carmine, a known agent used for measurement of ozone, we find ACK reacts reasonably fast with ozone. The ozone-ACK reaction rates do not seem to differ significantly when studied at different pH, meaning that the ozone reaction occurs with the double bond present in ACK. We are currently investigating the products formed and exploring the possibility of extending this method of ozone treatment to oxidize trace levels of ACK present in waste water and runoff waters. [Supported by grants from NSF (HRD 1043316) and US DoED (PO31B040030); * correspondence: rao_uppu@subr.edu.]
Polyimides are thermally stable at high temperatures, thus have wide variety of applications in aerospace industry. Carbon nano-tubes (CNT) reinforced polymer composites improve multifunctionalities such as structural and thermal properties. We prepared polyimide composites with 0 and 1 wt% CNTs. Three varieties of CNTs: Single Wall – functionalized, Double Wall – functionalized, and Double Wall – non-functionalized CNTs were used. The functionalization is performed by oxidation in acid followed by purification. Polyimides were prepared using BPADA, BAPP, and refluxing in anhydrous NMP followed by precipitation, cleaning, dissolving and dispersing CNT in NMP, and curing in a vacuum oven. High quality films of polyimides with and without CNTs were characterized using FT-IR, TGA, DSC, and Positron lifetime spectroscopy (PLS). The FT-IR spectra for all the samples confirmed the completion of polymerization to form polyimide in all the films. TGA curves showed weight loss with temperature in two stages: 180 - 300 °C with loss of ~ 15%, and 500 – 750 °C with weight loss of ~ 40% at 750 °C. The non-functionalizes CNT dispersed polyimide showed similar two-step behavior, but 80% weight remained at 750 °C. DSC curves of all polyimide samples showed two distinguishable endothermic peaks at around 90 °C and 200 °C. *Work partly performed at NASA Glenn Research Center – supported by NASA-CIPAIR grant. Authors thank Dr. Michael A. Meador, Dr. Marisabel Lebrón-Colón, and Mr. Daniel A. Scheiman at Glenn for their assistance.
A method was proposed to determine the effect of firing time on Yttria-Stabilized Zirconia (YSZ) microstructure for NOx sensing. A scanning electron microscope (SEM) was used to take images for calculation of the porous YSZ microstructure could be made. The SEM images were taken on 5mm X 10mm cut rectangular shape sensor with a porous YSZ structure. Sensor samples were fired over a temperature range of 950°C - 1050°C, for 1, 2, 4, 6 and 12 hours. The data collected was at 3.0kv 4.6mm x 45.0kv on a 1.00µm scale and 3.0kv 4.6mm x 60.0kv, on a 500nm scale. The resolution of the 1.00µm generated more accurate porosity estimates. The YSZ tape was about 50 - 53% porous when fired at temperatures ranged from 950°C - 1050°C. Analysis of Variance (ANOVA), indicated no interaction between temperature and time data, and the P-Values for temperature and time were 0.025907 and 2.27-5, respectively. The ANOVA analysis identified a slight distinction between the time and temperature data suggesting both affected porosity. Overall, it appeared that the firing temperature had a greater impact on porosity, in comparison to firing time. Supported by NSF EPSCoR Cooperative Agreement No. EPS-1003897 with additional support from the Louisiana Board of Regents.
RNA-SEQ ANALYSIS REVEALS THE DIFFERENTIAL EXPRESSION OF HOXA1 TARGET GENES IN MOUSE ES CELLS IN RESPONSE TO RETINOIC ACID.

X. Yi, *D. Refuge, and E. Martinez-Ceballos. Southern University and A&M College

The homeobox (Hox) family of transcription factors comprises important regulators of embryonic patterning and organogenesis. In mammals, the Hox genes are located in four separate chromosome clusters, Hoxa, Hoxb, Hoxc and Hoxd and their expression depends on their position in the chromosomal cluster: genes positioned at the 3’ end are expressed earlier and more anteriorly than 5’ end genes. In addition, Hox genes can be activated sequentially by retinoic acid (RA) in a manner that resembles their positions in the clusters, e.g. 3’ genes are activated by RA before 5’ genes. In vertebrate embryos, alterations of the normal pattern of Hox gene expression result in homeotic transformations and malformations. In mice, disruption of the Hoxa1 gene results in abnormal ossification of the skull, hindbrain, inner ear deficiencies, and neonatal death; however, little is known about the molecular events that occur downstream of Hoxa1 gene activation. In an attempt to elucidate the molecular mechanism of Hoxa1 action in mouse Embryonic Stem (ES) cells, gene expression profiling was carried out on Wild type versus Hoxa1−/− mouse ES cells using RNA-seq. Overall, transcriptome profiling revealed significant changes in the expression of 2842 genes. Of these, 1979 genes were upregulated by RA in Wt ES cells and 863 were downregulated in these cells as compared to Hoxa1−/− ES cells. The gene ontology of the differentially expressed genes is discussed further. These results provide an insight into the mechanism of Hoxa1 action in differentiating mouse ES cells. (Support: INBRE Program)
Poster #23

SERTRALINE, AN ANTIDEPRESSANT, INDUCES APOPTOSIS IN HEPATIC CELLS THROUGH THE MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY


Sertraline is used for the treatment of depression. Previously, we demonstrated that sertraline caused hepatic cytotoxicity and mitochondrial impairment. In the current study, we investigated and characterized molecular mechanisms of sertraline toxicity in human hepatoma HepG2 cells. Sertraline decreased cell viability and induced apoptosis. Sertraline activated the intrinsic checkpoint protein caspase-9 and caused the release of cytochrome c; this process was Bcl-2 family dependent because anti-apoptotic Bcl-2 family proteins were decreased. Pre-treatment of the HepG2 cells with caspase-3, -8, and -9 inhibitors reduced the release of LDH, indicating that sertraline-induced apoptosis is mediated by both intrinsic and extrinsic apoptotic pathways. Moreover, sertraline increased the expression of TNF and the phosphorylation of JNK, ERK1/2, and p38. In sertraline-treated cells, the induction of apoptosis and cell death was shown to be the result of activation of JNK, but not ERK1/2 or p38 in the MAPK pathway. Furthermore, silencing MAP4K4, the upstream kinase of JNK, attenuated both apoptosis and cell death caused by sertraline. Taken together, our findings suggest that sertraline induced apoptosis via activation of the TNF-MAP4K4-JNK cascade signaling pathway.
Our study assessed the effects of sub-chronic radio frequency radiation (RFR) exposure on the rat brain starting at the beginning of gestation and through postnatal day 49 (PND49), for a total of 10 weeks exposure. Male and female Sprague-Dawley rats were exposed to Global System for Mobile Communication (GSM) or Code Division Multiple Access Interim Standard 95 (CDMA) modulation at specific absorption rates (SAR) of 3, 6, or 9 W/Kg. After 10 weeks of exposure, brain tissues were harvested and either immediately frozen or fixed for subsequent HPLC, western blot, or histological analysis. A CDMA signal at 3 and 6 W/Kg induced HSP90 expression in the FC of female rats. GSM and CDMA at 6 and 9 W/Kg induced RAGE’s immunoreactivity and neurodegeneration, in male rat’s caudate nucleus (CN), with a CDMA signal at 9 W/Kg inducing identical changes in the frontal cortex (FC). In both sexes, a CDMA signal at 9 W/Kg increased dopamine (DA) and induced heat shock protein (HSP) 90 expression in the FC. This same signal also decreased DOPAC in the FC and increased heat shock protein HSP90 immunoreactivity in the CN of female rats. Overall, these data suggest that the effects of different types of signals emitted by mobile phones are dose-, region-, and gender-dependent. Further studies are necessary in order to elucidate the physiological implications of exposure (Supported by NCTR Protocol E02173).
PARTICULATE MATTER CONTAINING ENVIRONMENTALLY PERSISTENT FREE RADICALS INDUCE AhR-DEPENDANT CYTOKINE AND REACTIVE OXYDGEN SPECIES PRODUCTION IN EPITHELIAL CELLS

*VY Hebert1; SA Cormier2; R Reed2; W Backes2; TR Dugas1. 1Department of Pharmacology, Toxicology, and Neuroscience, LSU Health Sciences Center –Shreveport, LA, 2Department of Pharmacology, LSU Health Sciences Center-New Orleans, LA

Particulate matter (PM) is emitted during the combustion of fuels and wastes. Following combustion, aromatic compounds chemisorb to the surface of metal-oxide-containing PM, resulting in formation of surface-stabilized environmentally persistent free radicals (EPFR). Our studies showed that PM-containing EPFR redox cycle to produce reactive oxygen species (ROS), and after inhalation, EPFR induce pulmonary inflammation and oxidative stress. Our hypothesis was that EPFRs induce oxidant injury in pulmonary epithelial cells, and that the response is increased cytokine production. To test this hypothesis, we treated human bronchial epithelial cells with EPFR and measured ROS production, cell viability and cytokine levels. To test whether effects were dependent upon activation of the aryl hydrocarbon receptor (AhR) or oxidative stress, some cells were co-treated with an antioxidant, or AhR antagonist. In some cases, cells were transfected with a luciferase reporter for activation of the xenobiotic response element. Treatment of cells with EPFR induced an AhR activation that was partially dependent on ROS. Conversely, EPFR increased cellular ROS production in a manner that was partially regulated by AhR activation. Finally, EPFR-mediated cytokine release was dependent on both ROS and AhR activation. Thus, AhR activation may be a mechanism for altered epithelial cell function after EPFR exposure. Moreover, increased ROS production may be both a cause and an effect of AhR activation. Supported by NIEHS SRP.
Produkt # 26

SERTRALINE INDUCED ENDOPLASMIC RETICULUM STRESS IN HEPATIC CELLS

Lei Guo, Jiekun Xuan, Letha Couch, Si Chen

Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. FDA, Jefferson, AR 72079;

Sertraline is generally used for the treatment of depression. In our previous studies, we have demonstrated that sertraline caused mitochondrial impairment and apoptosis. In this study, we identified additional molecular mechanisms of sertraline’s toxicity using microarray analysis and confirmed further by biochemical and molecular analyses. HepG2 cells were exposed to sertraline and subjected to microarray analysis. Pathway analysis revealed that endoplasmic reticulum (ER) stress and MAPK pathway are among the significantly affected biological changes. Following the results of microarray study, we validated the increased expression of ER stress makers by real-time PCR and examined further by Western blot. The expression of ER stress molecules include CHOP, the hallmark of ER stress, were significantly increased. We also established in vitro systems monitoring ER stress response quantitatively and efficiently using Gaussia luciferase (Gluc) or secreted alkaline phosphatase (SEAP) as reporter. In these two reporter assays, sertraline showed inhibiting effects on the secretion of Gaussia and alkaline phosphatase. In addition, we also demonstrated that sertraline induced apoptosis coupled to ER stress and MAP4K4-JNK signaling pathway also participated in the regulation of sertraline induced ER stress.
Diethylene glycol (DEG) exposure poses potential risks to human health because of its widespread use in the industrial environment, as well as accidental exposures from contaminated products. In humans, the estimated lethal dose of DEG ranges up to 1.8 g/kg, at which dose (2 g/kg) rats do not show any toxicity, suggesting an increased susceptibility of humans to the acute effects of DEG. The purpose of this study was to determine a rodent model using dose response that would best mimic DEG toxicity in humans, and to provide information regarding rat strain differences in DEG exposure and toxicity. Male Wistar and Fischer-344 (F-344) rats were treated by oral gavage with water, 2 g/kg DEG, 5 g/kg DEG, or 10 g/kg DEG. Both rat strains treated with 10 g/kg had equivalent degrees of metabolic acidosis and renal toxicity. These effects were not observed at the lower doses, 2 g/kg and 5 g/kg, in either two strains. DEG-induced diuresis was observed in both strains at the 5 and 10 g/kg doses. Equivalent degrees of liver damage were also noted in both strains at the 10 g/kg dose, each having increased serum marker enzyme levels. These results indicate that both strains are equally sensitive to the toxic effects of DEG at the 10 g/kg dose, and show minor differences in strain sensitivity at the lower doses. To supplement the in vivo study, Wistar and F-344 rat proximal tubule (RPT) cells were exposed in culture to diglycolic acid (DGA). F-344 RPT cells displayed similar dose-dependent increases in necrotic cells death as did human PT cells, while Wistar RPT cells show less of an increase in DGA-induced cell death. Although both strains appear useful for in vivo DEG risk assessments, results indicate that the F-344 rat may be the more appropriate strain for interpretation of acute DEG effects in vitro.
Poster #28

MITOCHONDRIAL UNCOUPLING PROTEIN 2 IS UP-REGULATED IN HUMAN HEAD AND NECK, SKIN, PANCREATIC, AND PROSTATE TUMORS

W. Li, K. Nichols, C.A. Nathan and Y. Zhao

LSU Health Sciences Center in Shreveport, Shreveport, LA 71130

BACKGROUND: Mitochondrial uncoupling protein 2 (UCP2) uncouples electron transport from ATP production. UCP2 has been shown to play an important role in obesity and diabetes. Interestingly, studies have demonstrated that UCP2 is up-regulated in human colon cancer samples. OBJECTIVE: In order to study the role of UCP2 in human cancers, we detected the UCP2 protein level in various human tumor tissues. METHODS: Six types of human tumor and adjacent normal tissue samples were collected and analyzed by Western blot assays to detect the levels of UCP2. RESULTS: The results showed that in the human head and neck, skin, prostate, and pancreatic tumor samples examined, the protein levels of UCP2 were significantly higher in tumor tissues than that in the adjacent normal tissues. The protein levels of UCP2 was lower in non-small cell lung tumor tissues, which is marginal significant. CONCLUSIONS: Overexpression of UCP2 in certain tumors provides the rationale to speculate that UCP2 may promote tumor growth in these cancers. (Support: NIH Grant Number R21CA164218)
Withaferin A (WA) is a bioactive compound derived from Withania somnifera. The antitumor activity of WA has been well studied in human cancer models; however, its chemopreventive potential is unclear. In the present study, we used the skin epidermal JB6 P+ cells, a well-established model for tumor promotion, and demonstrated that WA suppressed the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA)-induced cell transformation and cell proliferation. Interestingly, TPA inactivated isocitrate dehydrogenase 1 (IDH1), which was reversed by WA. Similar results were also observed in mouse skin tissue. Therefore, we focused on metabolism as the potential mechanism of action. We found that mitochondrial functions were downregulated by TPA treatment, as indicated by reduced mitochondrial membrane potential, complex I activity and mitochondrial respiration. However, all of these downregulations were inhibited by WA. In addition, we examined the levels of α-ketoglutarate, a product of IDH1, and WA blocked its reduction upon TPA treatment. Finally, we detected the lactate level as a glycolysis marker, and WA suppressed its elevation caused by tumor promoter treatment. Altogether, these results suggest that WA might exert its chemopreventive activity via inhibiting not only oncogenic activation, but also IDH1 inactivation and mitochondrial dysfunction in early tumorigenesis.
Poster #30

COMPARING THE NEUROTOXICITY OF PROPOFOL AND KETAMINE IN RAT NEURAL STEM CELLS
F Liu*, N Sadovova, CM Fogle, TA Patterson, MG Paule, W Slikker Jr. and C Wang; Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079, USA.

Propofol and ketamine are widely used in pediatric anesthesia and analgesia. To evaluate their developmental neurotoxicity and examine underlying mechanisms, embryonic neural stem cells (NSCs) were used. NSCs were harvested from gestational day 14 rat fetuses and exposed on day 7 in culture to propofol at concentrations of 10, 50, 100, 300 and 600 µM, or ketamine at 1, 10, 100, and 500 µM ketamine in growth medium (GM) for 24 h or to 50 µM propofol or 10 µM ketamine in differentiation medium (DM). In GM propofol caused a dose-dependent reduction in NSC viability; while ketamine did not have this effect except at the very high concentration of 500 µM. At clinically-relevant concentrations in GM, propofol produced a dramatic increase in ROS generation and enhanced apoptosis as evidenced by an increase in Bax and in TUNEL-positive cells. Similar apoptotic effects were not observed at clinically-relevant dose of ketamine. No significant intracellular Ca²⁺ influx was detected when NSCs were stimulated with 50 µM NMDA in GM, suggesting there were no functional NMDA receptors expressed on NSCs in GM. In DM, most of the NSCs differentiated into neurons or glial cells. Differentiated neurons were identified using immuno staining with PSA-NCAM (a neuron-specific marker) and calcium influx stimulated with 50 µM NMDA suggested the existence of functional NMDA receptors. Propofol and ketamine significantly increased NSC ROS generation in DM. Propofol and ketamine caused neuronal damage but did not significantly affect glial cells. These observations suggest that ROS plays a key role in propofol and ketamine-induced neurotoxicity and that Ca²⁺ imaging and gene and protein arrays will be critical for defining associated mechanisms. In summary, an excitatory action of glutamate neurotransmission can be closely related to anesthetic-induced toxicity during development. Supported by NCTR/FDA E-7417
Poster #31

INCREASED ACCUMULATION OF 4-HYDROXYNONENAL ADDUCTS IN MALE GSTA4/PPARα DOUBLE KNOCKOUT MICE ENHANCES INJURY DURING EARLY STAGES OF ALCOHOLIC LIVER DISEASE.

K.Mercer1,2, N.Sharma1, E. Albano, E.3, T. Badger1,2, M. Ronis1,2, and D. Petersen3.

1Arkansas Children’s Nutrition Center and 2University of Arkansas for Medical Sciences, Little Rock, AR. 3 University A Avogadro of East Piedmont, Novara, Italy. 4University of Colorado Anschutz Medical Campus, Denver, CO.

In this study, we fed a Lieber-DeCarli ethanol (EtOH) liquid diet to male 129/SvJ glutathione S-transferase A4 knockout mice (GSTA4−/−) mice for 40 d. These mice lack the ability to metabolize lipid peroxidation products, particularly 4-hydroxynonenal (4-HNE). At sacrifice, we observed marked increases in 4-HNE adducts in liver sections, increased lipid accumulation, and mRNA expression of molecular markers of inflammation and fibrosis in the EtOH-treated GSTA4−/− mice compared to EtOH-treated wild type controls (p<0.05). Crossing the GSTA4−/− mice with the peroxisome proliferator-activated receptor-α null mice (PPARα−/−), which are predisposed to hepatic steatosis, had a significant impact on the degree of EtOH-mediated liver injury in the resulting double knockout (dKO) strain. EtOH-feeding of the dKO mice also resulted in significant elevation of hepatic lipid peroxidation adducts and auto-antibodies directed against these adducts when compared to EtOH-feeding of GSTA4−/−, PPARα−/− and wild type control mice (p<0.05). These findings highlight the importance of lipid peroxidation in alcohol-induced liver injury, and disease progression from steatosis to steatohepatitis and fibrosis. Funded in part by R01 AA009300 (DRP).
WHOLE EXOME SEQUENCING TO IDENTIFY GENETIC VARIANTS ASSOCIATED CLOPIDOGREL RESPONSES

*B. Ning¹, C-W. Chang¹, L. M. Yerges², D. Thierry-Mieg³, J. Thierry-Meig³, B. Green¹, Z. Su¹, J. R O’Connell², M. A. Pacanowski⁴, F. M. Goodsaid⁴, C. Morrison⁵, F. Lu⁵, X. Tan⁵, W. Tong¹, L. Shi¹ and A. R. Shuldiner²

¹National Center for Toxicological Research, FDA, Jefferson, AR, ²University of Maryland School of Medicine, Baltimore, MD, ³National Center for Biotechnology Information, NIH, Bethesda, MD, ⁴Center for Drug Evaluation and Research, FDA, Silver Spring, MD, ⁵SeqWright Inc., Houston, TX

From a genetically related Amish population, 1,893 adult participants were recruited and their DNA samples, social-economic status, dietary/life-style information and extensive phenotypic data were collected. The phenotype of clopidogrel responses was moderately heritable but the underlying genetic contributions have not been identified. We hypothesized that performing next-generation sequencing of the exomes may identify causative alleles for the phenotype. The selection of individuals for the exome-sequencing study was based on a standard genetic design using closely-related trios from the extremes of the population-response distribution (extremely high or extremely low responses) of the phenotypic trait. Candidate variants associated with the specific phenotype were identified by comparing genotypes of responders and non-responders. Based on these genetic and association studies, 15 SNPs associated with clopidogrel responses were chosen for further validation genotyping in an existing cohort consisting of approximately 1000 well-phenotyped individuals.
**Poster #33**

**KETAMINE-INDUCED NEURONAL TOXICITY IN AN EMBRYONIC NEURAL STEM CELL MODEL**

C Wang*, F Liu, N Sadovova, CM Fogle, T A Patterson, MG Paule, and W Slikker Jr.; Division of Neurotoxicology, National Center for Toxicological Research/FDA, Jefferson, AR 72079, USA.

Ketamine acts to block the NMDA receptor, involved in a variety of processes including: development and differentiation of the nervous system; and synaptic plasticity. To elucidate the underlying mechanisms associated with ketamine-induced neuronal toxicity and search for methods to prevent ketamine’s adverse effects on the developing brain, a neural stem cell model was utilized. Embryonic neural stem cells were harvested from gestational day 14 rat fetuses and on the 7th day in culture were exposed for 24 hrs to 1, 10, 100, and 500 µM ketamine in the growth medium (GM), and/or in differentiation medium exposed to either ketamine (10 µM), or ketamine plus acetyl-L-carnitine (10 µM) for 24 hrs, followed by a 24-hr withdrawal period. In the GM, no significant effect on LDH release was observed when neural stem cells were exposed to any of the four concentrations of ketamine. Consistently, mitochondrial viability (MTT uptake) was not significantly affected except at 500 µM, an extremely high concentration. In contrast, ketamine exposure in differentiation medium (DM) resulted in elevated generation of ROS as indicated by higher levels of DCFH production, and an increased number of TUNEL-positive cells. Ketamine-induced neuronal degeneration was observed using a neuron-specific marker (PSA-NCAM); no significant changes in astrocytes were detected. Co-administration of acetyl-L-carnitine significantly diminished ROS generation and provided protection of neurons from ketamine-induced cell death. No significant changes in Ca²⁺ influx were detected on neural stem cells, whereas Ca²⁺ influx was detected in neurons, after stimulation with 50 µM NMDA. These data suggest that ketamine exposure produces elevated ROS generation and neuronal cell death. Ketamine-induced neuronal damage is related to NMDA receptor expression and function. L-carnitine is a promising agent for reversing ketamine’s toxic effects on neurons during development. Supported by NCTR/FDA E-7417
Sevoflurane is a liquid halogenated ether inhalation anesthetic that is used to induce and maintain general anesthesia. It has been commonly used in surgical procedures for human infants and in veterinary and laboratory animal practice. While it is clear that general anesthetics cause neuronal cell death in rodent models when given repeatedly during the brain growth-spurt period, examination of their effects in a nonhuman primate model that more closely mimics the developing pediatric population is needed. Since levels of peripheral benzodiazepine receptor (PBR) increase in areas of neuronal injury following exposure to neurotoxicants, PBR is widely recognized as an important target for imaging using positron emission tomography (PET). In this study, $[^{18}F]$-radiolabelled phenoxyanilide ($[^{18}F]$-FEPPA) was used as an imaging agent for PBR. On PND 5/6, rhesus monkey neonates in the experimental group were exposed to 2.5% sevoflurane for 8 hours and control monkeys were exposed to room air only. On PND 6/7, $[^{18}F]$-FEPPA (56 MBq) was injected into the lateral saphenous vein of treated and control monkeys and microPET images were obtained over the next 2 hr. For follow-up, microPET scans were repeated for each monkey 1 and 3 weeks after exposure. Radiolabeled tracer accumulation in regions of interest in the frontal cortex and temporal lobe was converted into Standard Uptake Values. After each injection, the radiotracer was quickly distributed into the brains of both anesthetic-treated and control monkeys. On PND 6/7 the duration of tracer wash-out was prolonged in the anesthetic-treated animals. This prolonged wash-out was also observed in the brains of sevoflurane-treated monkeys 1 and 3 weeks post exposure. This preliminary study demonstrates that microPET imaging is capable of distinguishing differences in retention of $[^{18}F]$-FEPPA in different brain regions of nonhuman primates and suggests that this approach may provide a minimally-invasive biomarker of neuronal damage induced by sevoflurane.

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