30th Annual Meeting
of the
South Central Chapter of the
Society of Toxicology

“Advancing Toxicology for 30 Years”

November 1-2, 2012

Donald W. Reynolds Institute on Aging,
University of Arkansas for Medical Sciences,
Little Rock, AR
30th Annual Meeting of the South Central Chapter of the Society of Toxicology

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“Advancing Toxicology for 30 Years”

Thursday, November 1

Juanita’s, 614 President Clinton Ave., Little Rock, AR

6:30 – 9:00 PM Reception - Fajita Feast with cash bar
Sponsored by Center for Toxicology & Environmental Health LLC

Friday, November 2

Donald W. Reynolds Institute on Aging
University of Arkansas for Medical Sciences
Little Rock, AR

7:30 – 8:00 AM Continental Breakfast

8:00 – 8:15 AM Welcome & Opening Remarks

- Dr. Martin Ronis, Chair Local Organizing Committee
- Dr. Barbara Parsons, President SCC-SOT

8:15 – 9:15 AM Keynote Address – Dr. Russell S. Thomas
Senior Investigator and Director of the Institute for Chemical Safety Sciences at The Hamner Institutes for Health Sciences, Research Triangle Park, NC.

9:15 – 9:30 AM  Jone Corrales, University of Mississippi, “Transcriptome analyses of benzo[a]pyrene exposure in zebrafish embryos and larvae” (Platform Presentation 1)


9:45 – 10:00 AM  Sarah J. Blossom, University of Arkansas for Medical Sciences, “Postnatal trichloroethylene exposure induces behavioral changes and altered transsulfuration and transmethylation pathway metabolites in mouse cerebellum” (Platform Presentation 3)

10:00 – 10:15 AM  Peer W.F. Karmaus, St. Jude Children’s Research Hospital, “Differential effects of mTOR inhibition on the dendritic cell immune response” (Platform Presentation 4)

10:15 – 10:30 AM  Break

10:30 – 10:45 AM  Sridhar Jaligama, University of Louisiana-Monroe, “Effect of bone marrow inflammation in hexahydro-1-nitroso-3, 5-dinitro-1, 3, 5-triazine (MNX) induced myelosuppression in rats” (Platform Presentation 5)

10:45 – 11:00 AM  Arif Yurdagul Jr., Louisiana State University Health Sciences Center, Shreveport, “Matrix composition tunes the endothelial response to oxidized LDL between inflammation and apoptosis” (Platform Presentation 6)

11:00 – 11:15 AM  Jie Zhang, US FDA/National Center for Toxicological Research, “A novel biomarker, ROS/ATP ratio, accurately predicts drugs associated with serious drug induced liver injury (sDILI)” (Platform Presentation 7)

11:15 – 11:30 AM  Naeem K. Patil, University of Arkansas for Medical Sciences, “Early loss of renal manganese superoxide dismutase activity and mitochondrial respiration in the kidney during sepsis” (Platform Presentation 8)

11:30 – 11:45 AM  Orville Phillips, Southern University, “Characterizing the anti-cancer activity of Kola acuminata” (Platform Presentation 9)
11:45 – 12:00 PM Greg M. Landry, Louisiana State University Health Sciences Center, Shreveport, “Diglycolic acid induces human proximal tubule cell death by inhibition of succinate dehydrogenase and oxidative phosphorylation” (Platform Presentation 10)

12:00 – 12:15 PM Frank Booc, University of Mississippi, “Evaluating benzo[a]pyrene effects on steroidogenesis and reproduction” (Platform Presentation 11)

12:15 – 1:15 PM Lunch

1:15 – 1:30 PM Poster set-up

1:30 – 2:45 PM Poster Session (odd numbered posters attended)

2:45 – 4:00 PM Poster Session (even numbered posters attended)

4:00 – 4:30 PM Business Meeting
Awards Presentations
Adjournment
The South Central Regional Chapter of the Society of Toxicology would like to express its appreciation to the following organizations for financial support for its 2012 Annual Fall Meeting:

Xenometrics, LLC, Stillwell, KS

Charles River sponsored the Graduate Student Poster Presentation Award

Major support for the meeting ($1,000 or more) was provided by:

The Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR

Center for Toxicology & Environmental Health, L.L.C., Maumelle, AR sponsored the evening reception at Juanita’s

The Society of Toxicology provided funding to support student travel

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June 1, 2012 to May 31, 2012

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2012 SCC-SOT Annual Fall Meeting Local Organizing Committee

Dr. Martin Ronis, Chair, UAMS and Arkansas Children’s Hospital
Dr. Barbara Parsons
Dr. Tucker Patterson, NCTR
Dr. Wesley Gray, Southern University and A&M College
Dr. Kelly Mercer, UAMS
Dr. Meagan Myers, NCTR
Dr. Lei Guo
Russell S. Thomas, Ph.D.

Russell Thomas, Ph.D. is a Senior Investigator and Director of the Institute for Chemical Safety Sciences at The Hamner Institutes for Health Sciences, located in Research Triangle Park, North Carolina. Dr. Thomas also serves as Director of the Center for Genomic Biology and Bioinformatics.

Dr. Thomas has diverse research interests that range from basic research in cancer biology to applied research in chemical risk assessment. His current research interests focus on the development and application of genomic technologies and bioinformatic tools to toxicology and chemical risk assessment. Dr. Thomas completed his M.S. in radiation ecology and Ph.D. in Toxicology at Colorado State University. Following his doctoral studies, Dr. Thomas performed postdoctoral research in molecular biology and genomics at the McArdle Cancer Research Laboratory at the University of Wisconsin. Prior to joining The Hamner, Dr. Thomas worked in the biotech and biopharmaceutical industry.

Dr. Thomas has been recognized with many awards and honors, including: the Society of Toxicology Achievement Award (2009), Honorable Mention for Society of Toxicology Board of Publications Best Paper Award (2009), Best Papers Advancing the Science of Risk Assessment by the Risk Assessment Specialty Section (2007 & 2008). During his academic training, Dr. Thomas was awarded fellowships from the Colgate-Palmolive/Society of Toxicology, the National Cancer Institute, and Ciba-Geigy/Society of Toxicology. He has won awards for outstanding student presentations from Colorado State Cell and Molecular Biology and Mountain West Society of Toxicology, as well as the Kirke L. Martin Scholarship for Outstanding Graduate Student Research. Dr. Thomas was named a William J. Johnson Chemistry Scholar and received the Tabor College Divisional Science Award for Excellence. In 2011, he was selected for a Thought Leader Award by Agilent Technologies Inc. and the Agilent Technologies Foundation to support his work developing methods to predict drug-induced liver injury.
Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants that arise from incomplete burning of carbon-containing materials, such as diesel, gas, petroleum, coal, wood, and tobacco. PAHs, like benzo(a)pyrene (BaP), are carcinogenic, mutagenic and teratogenic. Here we present differences in the transcriptome of developing zebrafish after a parental BaP waterborne exposure followed by an offspring exposure. Adult zebrafish (2 males x 4 females, N=3 replicate tanks per treatment) were exposed to control or 42.0 ± 1.9 μg/L BaP for 7 days. Eggs were collected and raised in control conditions or continuously exposed to BaP until 3.3 and 96 hpf. Total RNA was extracted from a 50 embryo or 15 larva pool. Tagging and RNA-Seq libraries were constructed, and Illumina HiSeq-2000 was used for sequencing. To analyze data, RNA sequences were mapped using Galaxy and differential expression was done using EdgeR and DNASTAR ArrayStar. BaP exposure resulted in 227 and 264 up- and 47 and 666 downregulated genes at 3.3 and 96 hpf, respectively. The cyp family of genes and ahrr were upregulated while the tumor suppressor gene ext1b and developmentally important sox9b were downregulated. Molecular pathways impacted by BaP exposure and predicted with Metacore also will be presented. Supported NIEHS R21ES019940.
HYPERGLYCEMIA MITIGATES PARKINSON’S DISEASE: IN VITRO, ANIMAL MODEL, AND CLINICAL EPIDEMIOLOGIC EVIDENCE

Syed Z. Imam1,2,3,*, Mary Jo Pugh4, Zbigniew Binienda1, Eden Tareke1, Robert A. Clark3, William Slikker Jr.1, Balakuntalam Kasinath3, Syed F. Ali1, and Merle G. Paule1

1Division of Neurotoxicology, US FDA\National Center for Toxicological Research, Jefferson, AR; 2Department of Geriatrics, University of Arkansas for Medical Sciences, Little Rock, AR; 3Department of Medicine & 4Department of Epidemiology and Biostatistics, University of Texas Health Science Center, San Antonio, TX.

Background

Parkinson’s disease (PD) is a common neurodegenerative disorder featuring loss of dopaminergic neurons of the substantia nigra. Chronic systemic inflammations, as well as impaired mitochondrial metabolism, have been suspected of playing roles in the development of both PD and type 2 diabetes, and the possibility of shared pathophysiologic mechanisms by these two diseases has been put forth. However, reported data on associations between PD and diabetes mellitus (DM) are limited and conflicting.

Methods

A bivariate logistic regression model was used to quantify the relationships between PD and DM in a large national Veterans Health Administration cohort comprising inpatient, outpatient, and laboratory data from fiscal year 2005 (FY05; October 1, 2004 to September 30, 2005) through FY06 (October 1, 2005 to September 30, 2006) for veterans who were at least 65 years of age at the beginning of FY05. In addition, logistic regression models were used to predict PD using mean levels of HbA1c.

Results

In our animal studies, we found that elevated blood glucose levels mitigated dopaminergic damage in an animal model of PD. In addition, glucose protected against neurotoxin-mediated in vitro cytotoxicity in a human dopaminergic cell line. In the current epidemiological studies, we observed that the prevalence of PD was significantly decreased in patients with DM and that the age of onset of PD was significantly older in diabetics relative to non-diabetic controls. A further epidemiological evaluation of the prevalence and age of onset of PD in diabetic subjects stratified by HbA1c levels, as a surrogate for blood glucose levels over time, we found that mean HbA1c was inversely associated with the likelihood of new-onset PD.

Conclusion

This study provides strong epidemiologic evidence for an inverse association between DM and the risk of PD and suggests that hyperglycemia may protect against dopaminergic neuronal injury. Additional understanding of the mechanisms by which blood glucose levels impact neurodegenerative disease progression may open the way for novel therapeutic approaches to PD.
POSTNATAL TRICHLOROETHYLENE EXPOSURE INDUCES BEHAVIORAL CHANGES AND ALTERED TRANSSULFURATION AND TRANSMETHYLATION PATHWAY METABOLITES IN MOUSE CEREBELLUM


University of Arkansas for Medical Sciences College of Medicine, Arkansas Children’s Hospital Research Institute, Central Arkansas Veterans Healthcare System, Little Rock, AR

Previous studies have shown that continuous exposure throughout gestation and early life to environmentally-relevant doses of trichloroethylene (TCE) in the drinking water of MRL+/+ mice promoted adverse behavior associated with glutathione (GSH) depletion in the cerebellum indicating increased sensitivity to oxidative stress. Here we extend these findings to further characterize the impact of TCE exposure on redox homeostasis, biomarkers of oxidative stress, transmethylation metabolites, and global DNA methylation patterns in male mice exposed to water or two doses of TCE postnatally from birth until 6 weeks of age. Our results show that levels of GSH in cerebellum were lower compared to controls. In addition, increased biomarkers of oxidative stress and reduced methionine levels were also found in the TCE-exposed mice suggesting compromised cellular methylation. Indeed global DNA methylation patterns were significantly lower in TCE-exposed mice, but not controls. Unlike controls, mice exposed to TCE exhibited hyperlocomotor and increased exploratory behavior. These results point to the postnatal phase as a critical developmental stage where mouse cerebellum is a vulnerable target for the neurotoxic effects of TCE. Understanding the mechanisms of TCE-mediated neurotoxicity during sensitive windows of exposure is important in order to enhance mechanistic understanding of environmentally-related neurologic disorders in susceptible populations.

Support: National Institutes of Health (5R21ES017311-02) and the UAMS Translational Research Institute (National Institutes of Health UL1RR029884).
RAPAMYCIN INHIBITS THE MAMMALIAN TARGET OF RAPAMYCIN (mTOR)

mTOR is a protein kinase that acts as an evolutionarily conserved nutrient sensor. It exists in two distinct complexes: mTORC1 and mTORC2. mTORC1 is sensitive to rapamycin and controls cell growth, proliferation, metabolism, and autophagy. mTORC2, which is more resistant to rapamycin, is involved in actin organization.

DENDRITIC CELLS (DC) ARE CRUCIAL FOR IMMUNE RESPONSE

DCs play a central role in the immune system due to their ability to respond to innate immune stimuli and initiate T and B cell responses. In the present study, we investigated the effect of rapamycin on the ovalbumin (OVA)-induced airway hypersensitivity response as well as during innate activation of DC to elucidate the contribution of mTOR signaling in DC to inflammation. Rapamycin suppressed the maturation of inflammatory and tissue resident immune cell populations after OVA-induced airway hypersensitivity. Furthermore, rapamycin increased LPS-induced IL-12 levels in cultured DC in vitro. Using genetic ablation of Raptor, similar results were obtained as rapamycin treatment of DC in vitro. Overall, these results suggest that DC might contribute to rapamycin-induced inflammation involving the production of IL-12. (Support: St. Jude/ALSAC)
**PLATFORM #5**

**EFFECT OF BONE MARROW INFLAMMATION IN HEXAHYDRO-1-NITROSO-3, 5-DINITRO-1, 3, 5-TRIAZINE (MNX) INDUCED MYEOSUPPRESSION IN RATS.**

*S. Jaligama¹, V.M. Kale², M.S. Wilbanks³, E.J. Perkins³ and S.A. Meyer¹.

¹Dept of Toxicology, Univ of Louisiana-Monroe, Monroe, LA, ²College of Pharmacy, Roseman Univ of Health Sci, S. Jordan, UT, ³US Army Engineer Res and Dev Ctr, Vicksburg, MS.

MNX, an environmental nitroreduced product of munitions RDX, contaminates military sites. Our previous studies identified bone marrow (BM) and spleen as hematological targets of acute oral exposure to MNX in rats in the form of splenic hemosiderosis and loss of BM Granulocyte Macrophage Colony Forming Cells (GM-CFCs). To address whether late effects are due to persistence of early hematological effects or are late-onset due to required expression period, female Sprague-Dawley rats were orally gavaged with MNX from 0 to 94 mg/kg and different toxicological endpoints were evaluated over a time course at 2, 7, 10, 12, and 14d. Significant decrease in relative spleen weight and increased macrophage activity in splenic red pulp at 2d (≥ 47 mg/kg); and persistent splenic hemosiderosis at 2d (≥ 47 mg/kg), 7d (NOAEL 24 mg/kg) and 14d were observed. A significant increase in blood granulocytes and circulating levels of RANTES, a leukocyte chemokine, indicate that an acute inflammatory response occurs at 2d after exposure to MNX. Also, persistent BM macrophage infiltration in MNX (94 mg/kg) treated rat iliums (24h, 2d and 10d) and activation of NFkB signaling pathway in BM cells at 10d were evident. Further, significant increase in adherent BM mesenchymal stromal cell colonies constituting macrophages, endothelial cells and fibroblasts was observed in MNX (94 mg/kg) treated rats. Collectively, these data suggest that while splenic effects are early onset and persist for at least 14d; myelosuppression is delayed until 10d presumably due to development of inhibitory effects of preceding BM inflammation on myelopoiesis.

(Support: DoD/CDMRP, US Army Corps of Engineers)
PLATFORM #6

MATRIX COMPOSITION TUNES THE ENDOTHELIAL RESPONSE TO OXIDIZED LDL BETWEEN INFLAMMATION AND APOPTOSIS

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

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Oxidized LDL (oxLDL) accumulates at sites of early atherosclerosis and promotes endothelial cell proinflammatory gene expression and apoptosis. Since oxLDL promotes endothelial integrin activation, we examined the role of subendothelial matrix composition on the endothelial response to oxLDL. 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. Limiting integrin $\alpha_5\beta_1$ signaling is sufficient to inhibit oxLDL-induced inflammation. Ligation of $\alpha_5\beta_1$ on fibronectin enhances oxLDL-induced activation of the proinflammatory transcription factor NF-$\kappa$B, and NF-$\kappa$B inhibitors block oxLDL-induced inflammatory gene expression. In contrast to inflammation, adhesion to basement membrane proteins promotes oxLDL-induced apoptosis as measured by annexin V staining and caspase 3 activation. Taken together, these data suggest that the transition from basement membrane proteins to a transitional fibronectin-rich matrix switches the endothelial cell response to oxLDL from primarily pro-apoptotic to pro-inflammatory. (Support: Superior Toxicology Fellowship from the Louisiana Board of Regents grant LEQSF(2008-13)-GF-20 to AYJ and NIH R01 HL098435 to AWO).
A NOVEL BIOMARKER, ROS/ATP RATIO, ACCURATELY PREDICTS DRUGS ASSOCIATED WITH SERIOUS DRUG INDUCED LIVER INJURY (sDILI)

*J Zhang¹, A Suzuki², R Kelly¹, H Fang¹, A Li³ and W Tong¹

¹FDA/NCTR, Jefferson, AR, ²Central Arkansas Veteran Healthcare System, Little Rock, AR, ³In Vitro ADMET Laboratories LLC, Columbia, MD

Drug-induced liver injury (DILI) could cause serious clinical outcomes, such as acute liver failure. Prediction of such serious outcomes based on drug properties and/or patient’s risk factors remains a significant challenge. We recently developed an in vitro system using primary human hepatocytes to characterize biological properties of the marketed drugs with hepatotoxicity and to assess the accuracy of four biological endpoints, ATP content, reactive oxygen species (ROS), glutathione content (GSH), and caspase activity in predicting drugs associated with serious outcomes. Response of these biological outcomes to drug exposure was quantified by area under dose-response curves (AUC). Study drugs, 80 in testing and 72 in validation sets, were selected from the recently published unified list of drugs associated with hepatotoxicity (Drug safety 2010) and classified on whether to cause serious clinical/regulatory outcomes (defined by reported acute liver failure, boxed warning, and/or withdrawal) or not. We utilized the area under receiver operating characteristics curve (AUROC) to evaluate the prediction potential of biological endpoints for serious clinical/regulatory outcomes. ROC/ATP ratio yielded an excellent AUROC in both testing (0.92, P<0.0001) and validation set (0.88, P<0.0001). Among the drugs associated with the serious outcomes of DILI, ROS/ATP ratio could completely differentiate the hepatocellular injury drugs whether causing ALF or not. Further investigations are warranted to further delineate the mechanisms differentiating drugs which could/could not lead to serious clinical outcomes from hepatotoxicity.
Acute kidney injury (AKI) is a frequent complication of sepsis. Patients with sepsis complicated by AKI have a very high mortality rate (70%). A growing body of evidence suggests that mitochondrial injury might be playing a crucial role in the development of sepsis induced end organ damage. We hypothesize that renal mitochondrial dysfunction contributes significantly to the development of AKI during sepsis. To study this, we investigated the activities of mitochondrial manganese superoxide dismutase enzyme (MnSOD) and the respiratory complexes in a clinically relevant cecal ligation and puncture (CLP) murine model of sepsis. MnSOD activity was inhibited by nearly 50% at 4h and 18h after CLP, with no change in its protein levels. Interestingly, the MnSOD activity was recovered upon dithiothreitol treatment of the kidney lysate ex vivo, suggesting a role for its cysteine residues in inactivation. Also, the kidney mitochondrial complex II/III respiratory activity was decreased by nearly 25% and 50% at 6h and 18h respectively after CLP. In addition, mitochondrial targeted antioxidants including Mitoquinone and Mito-Tempo, both blunted the inhibition of complex II/III respiration studied at 18h after sepsis. Therefore, development of novel mitochondrial targeted anti-oxidant therapeutics might benefit to ameliorate kidney injury during sepsis.

(Support: NIH grant DK075991 and AHA grant 12PRE12040174)
Prostate cancer (PCa) is the leading cause of cancer death in American men in the United State, making it a major human health problem with a socio-economic burden in the millions of dollars. Despite rapid advancements in the early detection and treatment of PCAs over the past fifty years, there are no effective cures for this disease. Therefore, it is of paramount importance to develop new effective ways of slowing and/or preventing PCAs. Our laboratory has been studying the mechanism of the bioactive chemicals present in the Kola acuminate, a “cure-all” herbal natural medicine known to the Ettu people of Jamaica as obi or Bizzy nut. Based on the ethnobotanical and anecdotal data available on this natural product, we hypothesized that Bizzy nut contains one or more bioactive compounds that can modulate prostate biology. Using cell cytotoxicity, androgen receptor binding and androgen receptor gene expression as indices of androgenicity we found that the ether extract of Bizzy (Biz-2) contains compounds with potent anti-androgenic potential in both AR positive and AR negative prostate cells. Of the five extracts examined, Biz-2 was the only one that inhibited the proliferation of prostate cells (DU145 and LNCaP) in a concentration-dependent manner.

To validate the observed in vitro cytotoxicity and establish the specificity of this extract, we determined the growth inhibitory activity in vivo and in several different cancer cell lines. We observed that the Biz-2 extract displays cell specific cytotoxicity toward prostate cancer cells with no toxicity toward breast (MCF-7), lymphocyte (THP-1) or neuronal (GT1-7) cancer cells. Furthermore, Our current data shows that low doses of Biz-2 kill prostate cancer cells with no apparent effect on normal transformed prostate cells or displays any in vivo toxicity in mice. Next we examine the mechanism of action of Biz-2, induce cytotoxicity by examining LNCaP and DU145 cells exposed to Biz-2 for morphological signs of apoptosis, along with the expression of several apoptotic markers. In these experiments we found that Biz-2 was able to induce apoptotic cell death in prostate cancer cells. Surprisingly, Biz-2 was twice as potent in inducing apoptosis in the AR negative DU145 cells as in the AR positive LNCaP cells. This study has demonstrated that that an ether extract of Bizzy nut (Biz-2) can modulate prostate cancer cell function. (Work was supported by a NIH grant, Grant No. 3P20RR016456-08S1)
DIGLYCOLIC ACID INDUCES HUMAN PROXIMAL TUBULE CELL DEATH BY INHIBITION OF SUCCINATE DEHYDROGENASE AND OXIDATIVE PHOSPHORYLATION

*GM Landry, CL Dunning, TV Dupre, MJ Hitt, KE McMartin.

Department of Pharmacology, Toxicology & Neuroscience, Louisiana State University-Health Sciences Center, Shreveport, LA

Diethylene glycol (DEG) is an organic solvent used in common consumer products allowing the increased risk for exposure. DEG metabolism produces two primary metabolites, 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA). DGA, not DEG or 2-HEAA, produces proximal tubule cell necrosis leading to acute renal failure, the hallmark of DEG poisoning. Studies were designed to assess whether the mechanism for DGA-induced cytotoxicity involves disruption of cellular metabolic processes resulting in mitochondrial dysfunction. DGA induces severe ATP depletion in human proximal tubule (HPT) cells that occurs prior to significant cell death. HPT cells pretreated with increasing DGA concentrations showed significant decreases in oxygen consumption suggesting that DGA acts as an oxidative phosphorylation inhibitor, rather than a mitochondrial electron transport chain uncoupler. Co-incubation of DGA with the antioxidant, α-tocopherol significantly reduced DGA-induced reactive oxygen species (ROS) formation, but had no effect on ethidium homodimer uptake or lactate dehydrogenase release, two measures of necrotic cell death, suggesting that ROS production is not a cause, but a consequence of DGA-induced cell death. DGA treatment also significantly and preferentially inhibits succinate dehydrogenase activity, but has no effect on other citric acid cycle enzyme activities. These results indicate that DGA produces proximal tubule cell dysfunction by specific inhibition of mitochondrial-mediated processes resulting in decreased energy production, oxygen utilization, and ultimately cellular necrosis.
EVALUATING BENZO[A]PYRENE EFFECTS ON STEROIDOGENESIS AND REPRODUCTION

* F. Booc¹, C. Thornton¹, X. Fang¹, A. Lister², and D. MacLatchy², K.L. Willett¹

¹Department of Pharmacology and Environmental Toxicology Research Program, School of Pharmacy, University of Mississippi, University, MS. USA
²Department of Biology, Wilfrid Laurier University, Waterloo, CA

Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon (PAH) that has been implicated in modulating aromatase enzyme function. This effect has potential to interrupt normal reproductive function by causing imbalances in homeostatic androgen and estrogen levels. The aim of this study was to use a fish model, Fundulus heteroclitus, in order to assess whether BaP causes a significant change in steroid concentrations that could negatively alter additional reproductive biomarkers. Adult fish were exposed to waterborne BaP concentrations of (0, 1 or 10 μg/L) for 28 days. Males and females were combined for the second half of the exposure (days 14-28) in order to quantitate egg production and fertilization rates. BaP exposure did significantly reduce male gonad somatic index (GSI) and egg fertilization at 10 μg/L. Additionally, testosterone in the males was significantly reduced at 10 μg/L, and estradiol concentrations in the females was significantly reduced at 1 and 10 μg/L. Sperm concentrations, male liver somatic index (LSI), and female GSI and LSI were not altered. BaP exposure at these environmentally relevant concentrations caused negative alterations to both molecular and phenotypic biomarkers associated with reproduction. Our next goal is to assess if these parental effects will cause permanent changes in subsequent generations of progeny. (Supported by NIEHS R03 ES018962)
1. **EVALUATION OF APOPTOSIS AND AUTOPHAGY INDUCED BY NOVEL mTOR AND PROTEASOME INHIBITORS IN HUMAN RENAL ANGIOMYOLIPOMA CELLS.** *AP. Clark¹, L. Lu², A. Nicole White³, Brian J. Siroky⁴, John J. Bissler⁵, Department of Chemistry¹, Southern University A&M College Baton Rouge LA, Division of Nephrology and Hypertension², The Division of Rheumatology³, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio.

2. **EFFECT OF Diallyl TRISULFIDE (DATS) ON MIEN1 MEDIATED PROSTATE CANCER PROGRESSION.** L. Brown¹, P. Chaudhary², and J.K. Vishwanatha³, ¹Department of Chemistry Southern University A&M College, ²Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, Texas.

3. **SWEET TASTANTS INFLUENCE INSULIN SYNTHESIS IN B CELLS IN ISLETS OF LANGERHANS.** A.C. Harrison¹, K. S. Gravenstein², Chee W. Chia³, Josephine M. Egan²³, Department of Chemistry Southern University and A & M College¹, Clinical Research Branch², Laboratory of Clinical Investigation³; National Institute on Aging (NIA) Intramural Research Program, Baltimore, Maryland.

4. **HYPERTENSION IS ASSOCIATED WITH AN INCREASE IN THE NUMBER OF CATECHOLAMINERGIC NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT.** S. Edwards¹, C. Yuan, and S. Mifflin², ¹Department of Biology, Southern University A & M College, Baton Rouge, La ²Department of Integrative Physiology, UNT Health Science Center, Fort Worth, TX.

5. **KINETICS FOR THERMAL INACTIVATION OF SHIGA-LIKE TOXIN 2 IN MILK.** R. Nshuti¹, W.H. Tolleson², ¹Philander Smith College, Little Rock, AR; ²National Center for Toxicological Research, Jefferson, AR.

6. **SOY ISOFLAVONE PHYTOESTROGEN, GENISTEIN, DOES NOT PROTECT AGAINST ALCOHOL INDUCED OSTEOPOROSIS IN MALE MICE.** *C. Yang¹ K.E. Mercer¹, L.J. Suva² T.M. Badger¹ and M.J. Ronis³, ¹Arkansas Children’s Nutrition Center* and the ²University of Arkansas for Medical Sciences, Little Rock, AR.

7. **ROBOTIC MODELING AND THE ENHANCE OF THE STEM SUBJECTS.** *K. Graham¹ and T. Green², ¹Department of Electrical Engineering Southern University A&M College, Baton Rouge, LA, and ²Scientific Research, Jackson, MS.

8. **COMPARISON OF AVAILABLE “FEED STOCK” FOR BIODIESEL PRODUCTION.** *SN Jones-Butts¹ and K. Crosby², Department of Chemistry¹, Department of Civil Engineering² Southern University A&M College, Baton Rouge, LA.

10. **GENE EXPRESSION CHANGES IN THE VISUAL CENTERS OF THE BRAIN IN BALB/cJ MICE AFTER OPTIC NERVE CRUSH.** A. Robinson¹, T. Putliwala², C. McDowell², Y. Liu², T. L. Casavant³, B. Faga³, D. Thole³, R. J. Wordinger², T. A. Braun³, A. F. Clark², ¹Dept. of Biology, Southern University and A & M College, Baton Rouge, LA ²Dept. Cell Biology & Anatomy, NTERI, UNTSC, Ft. Worth, TX ³Dept. Bioinformatics and Computational Biology, UIOWA, Iowa City, IA.

11. **AN EVALUATION OF THE PKM2 INHIBITORS, COMPOUND 3 AND SHIKONIN, IN NEUROBLASTOMA.** *C. Collins, Y. Zhao, and D. Robbins, LSU Health Shreveport, Department of Pharmacology, Toxicology & Neuroscience, LSU Shreveport, LA.

12. **ASSESSMENT OF GENE EXPRESSION LEVELS IN THE PROSTATE OF RATS DOSED ORALLY WITH BISPHENOL A FROM GESTATION DAY 6 UNTIL POSTNATAL DAY 4.** *R Prabhu, KB Delclos, L Camacho, Division of Biochemical Toxicology, National Center for Toxicological Research, Food and Drug Administration, AR.

13. **GENE EXPRESSION AND DNA METHYLATION STATUS IN THE PROSTATE OF RATS DOSED ORALLY WITH BISPHENOL A FROM GESTATION DAY 6 UNTIL POSTNATAL DAY 90.** *L Camacho¹, I Koturbash², SM Lewis³, M Vanlandingham¹, IP Pogribny¹, KB Delclos¹, ¹Division of Biochemical Toxicology and ³Office of Scientific Coordination, National Center for Toxicological Research, Food and Drug Administration, AR; ²Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, AR.

14. **FLIPT1 (SLC22A15) MEDIATES THE UPTAKE OF THE ANTICANCER ADENOSINE ANALogue, F-ara-A.** J. Xuan¹, S.W. Yee², J.E. Shima², A. Schlessinger², E. Geier², A. Sali², K. M. Giacomini²,³ ¹Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, AR; ²Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, CA; ³Department of Pharmaceutical Chemistry, and California Institute for Quantitative Biosciences, University of California, San Francisco, CA.
15. INTEGRATING LOCAL COMMUNITIES IN THE HEALTH RISK ASSESSMENT PROCESS FOLLOWING THE DEEPWATER HORIZON OIL SPILL—A FOCUS ON VIETNAMESE-AMERICANS. *M.J. Wilson¹, S. Frickel², D. Nguyen³, J.L. Howard¹, B.R. Simon⁴, and J.K. Wickliffe¹.¹ Department of Global Environmental Health Sciences, School of Public Health and Tropical Medicine, Tulane University, New Orleans LA. ²Department of Sociology, Washington State University, Pullman WA. ³Mary Queen of Vietnam Community Development Corporation, New Orleans LA. ⁴Department of Biomedical Sciences, School of Medicine, Tulane University, New Orleans LA. ⁵Tulane Cancer Center, Tulane University, New Orleans LA.

16. REDUCED MANGANESE SUPEROXIDE DISMUTASE IN KIDNEY: TRIGGER FOR DISRUPTED MITOCHONDRIAL HOMEOSTASIS? *N Parajuli, LA MacMillan-Crow, Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences

17. EVALUATION OF PERCHLORATE INTAKE ON MATERNAL AND FETAL SERUM T₄ LEVELS USING A BBDR MODEL. *A. A. Lumen¹,³, D. R. Mattie² and J. W. Fisher³, ¹HJF, Jefferson, AR, ²711 HPW/RHDJ, WPAFB, OH, ³FDA/NCTR, Jefferson, AR.

18. INHIBITION OF ADIPOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS BY TIO2 NANOPARTICLES. Y. Zhang¹*, L. Guo², L. Yu², Y. Gao², Y. Jones¹, A. Keasling¹, B. Green², D. Hansen², A. Inselman², L. Shi², P.C. Howard¹, B. Ning², ¹NCTR/ORAN Nanotechnology Core Facility, Office of Scientific Coordination, ²Division of Systems Biology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR.

19. QUANTITATIVE ASSESSMENT OF [¹⁸F]-FEPPA UPTAKE AS A BIOMARKER OF ANESTHETIC-INDUCED NEUROTOXICITY. X Zhang¹, MG Paule¹, GD Newport¹, S Liu¹, MS Berridge², SM Apana², W Slikker, Jr.¹ and C Wang¹*,¹ Division of Neurotoxicology, NCTR, FDA, Jefferson, AR, ²3D Imaging, LLC, Little Rock, AR.

20. MUTAGENIC POTENCY OF FIVE CHEMICALS CONTAINED IN CIGARETTE SMOKE. *X. Guo, H. Lin, S.L. Dial, P. Richter, M.M. Moore and N. Mei, Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, Jefferson, AR.

21. IN VITRO GENOTOXIC EFFECTS OF GINKGO BILOBA EXTRACT AND ONE OF ITS MAJOR COMPONENTS, QUERCETIN. Lin H, Guo X, Dial SL, Manjanatha MG, Moore MM, Mei N, Division of Genetic and Molecular Toxicology, NCTR/FDA, Jefferson, AR.

23. DETERMINATION OF ION-RELEASE KINETICS AND TOXICITY IN AN ENVIRONMENTALLY RELEVANT AQUEOUS MEDIUM. Harmon AR, Kennedy AJ, Poda AR, Bednar AJ, Steevens JA, US Army Engineer Research and Development Center, Environmental Laboratory, Vicksburg, MS.

24. ROS/REDOX SIGNALING REGULATES BONE TURNOVER IN AN AGE-SPECIFIC MANNER IN FEMALE MICE. *K.E. Mercer1,2, R., L.J. Suva3, T.M. Badger1,2, J-R Chen1,2 and M.J. Ronis1,2, 1Arkansas Children’s Nutrition Center, 2University of Arkansas for Medical Sciences, Little Rock, AR.

25. VITAMIN D SUPPLEMENTATION PREVENTS HYPOCALCEMIA AND CORTICAL BONE LOSS ASSOCIATED WITH CHRONIC ALCOHOL FEEDING IN FEMALE MICE. K. Mercer 1,2, R.A. Wynne1, O.P. Lazarenko1, C.K. Lumpkin3, W.R. Hogue,2, L.J. Suva2, J.R. Chen21,2, T. M. Badger,1,2, *M.J. Ronis1,2. 1Arkansas Children’s Nutrition Center, 2University of Arkansas for Medical Sciences Little Rock, AR.

26. SILENCE OF SOX2 INDUCES APOPTOSIS THROUGH BOTH MITOCHONDRIA AND DEATH RECEPTOR SIGNAL PATHWAY BY ACTIVATING THE RAS/MAPK SIGNALS IN HUMAN LUNG CANCER CELLS. *S.Chen1,2, D.Lv1, R. A. Reisfeld3, N.Li1 and R.Xiang1, 1Division of Biochemical Toxicology, National Center for Toxicological Research (NCTR), Food and Drug Administration (FDA), Jefferson, AR, 2School of Medicine, Nankai University, Tianjin, China, 3Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, CA.

27. IN VIVO DEMONSTRATION OF FLUORO-TURQUOISE CONJUGATED GELATIN FOR THE DEMONSTRATION OF ENDOTHELIAL CELLS IN THE BRAINS OF HEALTHY AND KAニック ACID EXPOSED RATS. Sumit Sarkar, James Raymick and Larry Schmued, Division of Neurotoxicology, FDA/NCTR, Jefferson, AR.

28. IDENTIFICATION OF GENETIC VARIANTS IN DISEASE RISKS AND DRUG RESPONSES BY NEXT-GENERATION SEQUENCING. *B. Ning1, C-W. Chang1, L. M. Yerges2, D. Thierry-Mieg3, J. Thierry-Mieg3, 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.

29. DIGLYCOLIC ACID, THE NEPHROTOXIC METABOLITE OF DIETHYLENE GLYCOL, INHIBITS INTRACELLULAR 14C-SUCCINATE UPTAKE IN HUMAN PROXIMAL TUBULE CELLS IN VITRO. *TV Dupre1,2, GM Landry1, and KE McMartin1, 1Department of Pharmacology, Toxicology, and Neuroscience, LSU Health Sciences Center, Shreveport, LA, 2University of Louisiana at Monroe Toxicology Program, Monroe, LA.
30. TRICHLOROETHYLENE INDUCES EPIGENETIC ALTERATIONS IN CD4+ T CELLS, *K.M. Gilbert, S.J. Blossom and C. Cooney, Arkansas Children’s Hospital Research Institute, UAMS, Little Rock, AR; Central Arkansas Veterans Healthcare System, Little Rock, AR.

31. SIZE DEPENDENT ANTIBACTERIAL EFFECTS OF SILVER NANOPARTICLES (AGNP) ON BACTERIAL SPECIES OF THE GASTROINTESTINAL (G.I.) TRACT. *M. Imam1, S. Khare 2, A. Paredes3, and M. Boudreau1, 1Division of Biochemical Toxicology, 2Division of Microbiology, and 3Office of Scientific Coordination, National Center for Toxicological Research, Jefferson, AR.

32. DEVELOPMENT OF DOXORUBICIN-INDUCED CHRONIC CARDIOTOXICITY IN THE B6C3F1 MOUSE MODEL, Varsha Desai1*, Eugene Herman2, Carrie Moland1, William Branham1, Sherry Lewis3, Kelly Davis4, Nysia George5, Susan Kerr6, James Fuscoe1, 1Division of Systems Biology, NCTR, Jefferson, AR. 2Division of Applied Pharmacology Research, CDER, Silver Spring, MD. 3Office of Scientific Coordination, 4Toxicologic Pathology Associates, 5Division Bioinformatics and Biostatistics, NCTR, Jefferson, AR. 6Arkansas Heart Hospital, Little Rock, AR.

33. BENZO[α]PYRENE DIETARY EXPOSURE EFFECTS ON REPRODUCTIVE SUCCESS AND F1 DEVELOPMENT IN ZEBRAFISH, *M. White, C. Thornton, J. Corrales, K. Mislan, K.L. Willett, Environmental Toxicology Research Program, University of Mississippi, University, MS.

34. METABOLIC EVALUATING PROTECTIVE EFFECTS OF GREEN TEA EXTRACT ON ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN MICE. *Lu, Yihong1,2, Sun, Jinchun1, Yang, Xi1, Greenhaw, James1, Salminen, Willie1,3, Mendrick, Donna L.1, Beger, Richard D.1, 1 Division of Systems Biology, National Center for Toxicological Research, US FDA, Jefferson, AR, 2Jiangsu Institute for Food and Drug Control, Jiangsu, China3, PAREXEL International, Boston, MA.

35. ALPHA-SYNUCLEIN-MEDIATED ACTIVATION OF C-ABL AND DOPAMINE DEPLETION IN DOPAMINERGIC NEOURAL CELLS TREATED WITH IRON-OXIDE NANOPARTICLES OR METHAMPHETAMINE. *S.M. Lantz1*, Z. Binienda1, L. Mohammed Saeed1, B. Robinson1, M.G. Paule1, A.S. Biris2, S.F. Ali1, and S.Z. Imam1, 1Division of Neurotoxicology, US FDA/NCTR, Jefferson, AR. 2Nanotechnology Center, University of Arkansas at Little Rock, AR.

36. POTENTIAL REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF OXYBENZONE – A DOSE-FINDING STUDY. *A.L. Inselman1, N. Nakamura1, G. White1, W. Harrouk2, B. McIntyre3, P. Foster2, and D.K. Hansen1, 1NCTR, U.S. FDA, Jefferson, AR; 2CDER, U.S. FDA, Silver Spring, MD; 3NTP, NIEHS, NIH, Research Triangle Park, NC.
37. INCREASED MUCUS PRODUCTION AND HISTOPATHOLOGICAL GILL ALTERATIONS AFTER EXPOSURE TO NANOSILVER AND SILVER NITRATE. *A.D. Hawkinsa, C. Thorntona, A. Harmonb, A.J. Kennedyb, J. Steevensb, and K. L. Willetta, a ETRP, University of Mississippi, University. b US Army ERDC, Vicksburg, MS.

38. EVALUATING THE EFFECTS OF PENICILLIN TREATMENT ON THE URINE AND PLASMA METABOLOMES OF SPRAGUE-DAWLEY RATS. Sun, Jinchun1, Schnackenberg, Laura K.1, Yang, Xi1, Greenhaw, James1, Salminen, William1,2, Mendrick, Donna L.1, Beger, Richard D.1, 'Division of Systems Biology, National Center for Toxicological Research,US FDA, Jefferson, AR, 2PAREXEL International, Boston, MA, USA

39. EFFECTS OF BENZO[A]PYRENE ON EARLY ZEBRAFISH DEVELOPMENT. K. Alharthy, F. Booc, J. Corrales, C. Thornton, K.L. Willett, Department of Pharmacology and ETRP, School of Pharmacy, University of Mississippi, University, MS.

40. PREVALENCE OF KRAST MUTANT TUMOR SUBPOPULATIONS. *P.B. McKinzie, M.B. Myers, K.L. McKim, Y. Wang, F. Meng, B.L. Parsons, Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, US FDA, Jefferson, AR

41. AGE- AND SEX-RELATED ALTERATIONS IN RENAL GLOBAL DNA METHYLATION DURING THE LIFE CYCLE OF RATS. Tao Han, Carrie L. Moland, Josh Kwekel, Varsha G. Desai, and James C. Fuscoe, Personalized Medicine Branch, Division of Systems Biology, National Center for Toxicological Research, U.S. FDA, Jefferson, AR.

42. EFFECT OF FETAL EXPOSURE TO OXYBENZONE ON TESTES DEVELOPMENT IN MALE RATS. *N. Nakamura, A.L. Inselman, G.A. White, C.-W. Chang and D.K. Hansen, National Center for Toxicological Research, FDA, AR

43. ROLE OF NEURAL STEM CELL ACTIVITY IN POSTWEANING DEVELOPMENT OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA IN RATS. 1Z. He, 2S.A. Ferguson, 2L. Cui, 2L. J. Greenfield, Jr., 1M.G. Paule, 1Division of Neurotoxicology, NCTR FDA; 2Department of Neurology, UAMS.

45. EVALUATION OF WILD YAM (Dioscorea villosa) ROOT EXTRACT AS A POTENTIAL EPIGENETIC AGENT IN BREAST CANCER CELLS. *P. Aumsuwan 1, S.I. Khan 2,3, I. A. Khan 2,3, L.A. Walker 1,2, A. K. Dasmahapatra 1,2, 1 Department of Pharmacology, 2 National Center for Natural Product Research, 3 Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS.

46. NICOTINE-INDUCED TOXICITY IN RAT EMBRYONIC NEURAL STEM CELLS. Fang Liu, Natalya Sadovova, Charles Fogle, Merle G. Paule, William Slikker, Jr., and Cheng Wang, Division of Neurotoxicology, National Center for Toxicological Research (NCTR)/FDA, Jefferson, AR.

47. A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR BISPHENOL A IN RATS AT DIFFERENT DEVELOPMENTAL AGES. *XX Yang, DR Doerge, and JW Fisher, Food & Drug Administration, National Center for Toxicological Research, Division of Biochemical Toxicology, Jefferson, AR.

48. MAGNETIC RESONANCE IMAGING AND SPECTROSCOPIC MARKERS OF KAINIC ACID-INDUCED EXCITOTOXICITY IN THE RAT BRAIN. *S. Liachenko1, J.Ramu1, M.G.Paule1, L.Schmued1, J.Hanig2, 1NCTR/FDA, Jefferson, AR, 2CDER/FDA, White Oak, MD.

49. EFFECTS OF CROCETIN AND SAFRANAL, CONSTITUENTS OF SAFRRON, IN 22Rv1 PROSTATE CANCER CELLS. * F. F. Albaqami and K. L. Willett, Department of Pharmacology, School of Pharmacy, University of Mississippi, MS.

50. L-CARNITINE AMELIORATES PROPOFOL-INDUCED TOXICITY IN RAT EMBRYONIC NEURAL STEM CELLS. Cheng Wang, Fang Liu, Charles Fogle, Merle G. Paule and William Slikker, Jr, Division of Neurotoxicology, National Center for Toxicological Research (NCTR)/FDA, Jefferson, AR.

51. PKM2 INHIBITOR SHIKONIN SUPPRESSES TPA-INDUCED MITOCHONDRIAL MALFUNCTION AND PROLIFERATION OF SKIN EPIDERMAL JB6 CELLS. *Wenjuan Li1, Joan Liu2, Yunfeng Zhao1, 1Department of Pharmacology, Toxicology & Neuroscience, LSU Health Sciences Center in Shreveport, Shreveport, LA; 2 Caddo Parish Magnet High School, Shreveport, LA.

52. NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS INDUCE PREMATURE SENESCENCE IN AORTIC ENDOTHELIAL CELLS. Valeria Y. Hebert, Kate Slaybaugh, Corie N. Robinson, Stephen Xue, Danicia Hayes, Mitzi C. Glover, and Tammy R. Dugas, Department of Pharmacology, Toxicology & Neuroscience, LSU Health Sciences Center, Shreveport, LA.
53. SHORT-TERM AND CHRONIC EXPOSURES OF CIGARETTE SMOKE CONDENSATE (CSC) INDUCE DIFFERENTIAL EXPRESSION AND PROMOTER METHYLATION PROFILES OF CRITICAL GENES INVOLVED IN LUNG CANCER: In Vitro ANALYSIS OF POTENTIAL HARM OF TOBACCO SMOKE IN LUNG. Beverly Word, Lascelles E. Lyn-Cook, Jr., Beverly Word, BiBi Mwamba, Beverly Lyn-Cook, and George Hammons, FDA/NCTR, Jefferson, AR.

54. INFLUENCE OF AGE AND SEX ON MITOCHONDRIA-RELATED GENE EXPRESSION IN RAT HEART. *V. Vijay, T. Han, C.L. Moland, J.C. Kwekel, J.C. Fuscoe, V.G. Desai, Personalized Medicine Branch, Division of Systems Biology, NCTR, FDA, Jefferson, AR.

55. EFFICACY AND SAFETY OF CHRONIC ANTI-(-)-METHAMPHETAMINE MAB7F9 TREATMENT OF (+)-METHAMPHETAMINE (METH) INDUCED LOCOMOTOR ACTIVITY IN RATS. *M.D. Hambuchen, D. Rüedi-Bettschen, and SM Owens, Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, AR.

56. KIDNEY miRNAs SHOW AGE AND SEX DIFFERENCES IN EXPRESSION DURING THE RAT LIFE CYCLE. *JC Kwekel, VG Desai, T Han, CL Moland, and JC Fuscoe, Personalized Medicine Branch, Division of Systems Biology, National Center for Toxicological Research, U.S. FDA, Jefferson, AR.

57. IN VIVO EFFECTS OF ABUSED “BATH SALT” CONSTITUENT 3,4-METHYLENEDIOXYPYROVALERONE (MDPV) IN MICE: DRUG DISCRIMINATION, THERMOREGULATION, AND LOCOMOTOR ACTIVITY. William E. Fantegrossi¹, Brenda Gannon¹, Sarah M. Zimmerman¹, and Kenner C. Rice², ¹Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, ²Drug Design and Synthesis Section, Chemical Biology Research Branch, NIDA and NIAAA, Bethesda, MD.

58. MITOCHONDRIAL BIOGENESIS AND MITOPHAGY FOLLOWING MANGANESE SUPEROXIDE DISMUTASE KNOCKDOWN. *Akira Marine, Lee Ann MacMillan-Crow, Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR.

59. DEVELOPMENTAL EXPOSURE TO CHLORPYRIFOS INCREASES ACCUMULATION OF THE ENDOCANNABINOID ANANDAMIDE IN THE BRAIN IN THE ABSENCE OF CHOLINESTERASE INHIBITION. R. L. Carr; C. A. Nail; L. C. Mangum; M. K. Ross, Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.
60. **Asian Ginseng (Panax Ginseng) Potentiates Ethanol-Induced Cardiovascular Dysfunction in Medaka Embryogenesis (Oryzias Latipes).** *M.H. Haron\(^1,2\), L.A. Walker \(^1,2\), I.A. Khan \(^1\), and A.K. Dasmahapatra \(^1,2\),  \(^1\)National Center for Natural Product Research, \(^2\)Department of Pharmacology, University of Mississippi, University, MS.

61. **Serotonin Signaling After Acute Diisopropylfluorophosphate (DFP) Intoxication: Modulation by Endocannabinoids?** *R. Maples and C. Pope, Vet Med, Oklahoma State University, Stillwater, OK.*
Tuberous Sclerosis Complex is a tumor predisposition syndrome caused by mutations in TSC1 or TSC2, which leads to the formation of angiomyolipomas. Our lab previously discovered that TSC2-deficient human renal angiomyolipoma cells were more sensitive to proteasome, MG-132 compared to TSC2-rescued cells. Thus, we postulated that MLN2238 (Millennium Pharm.), a novel proteasome inhibitor, will kill TSC2-deficient angiomyolipoma cells more effectively than TSC2-rescued cells. Furthermore, the novel mTOR inhibitor Torin2, which is also thought to more effectively induce autophagy compared to other available mTOR inhibitors, will enhance this effect. In this study, we used Crystal Violet Cell Viability Assay to measure cell viability. We used Western Blot and ImageStream X image-based flow cytometry to measure and identify apoptotic and/or autophagic cells. Both the viability assay and western blot indicated that the TSC2-deficient cells were more sensitive to MLN2238 compared to the TSC2-rescued cells. The combination of an mTOR inhibitor with MLN2238 was more effective in reducing cell viability based on the viability assay. However, MLN2238 was more effective in inducing apoptosis alone. Torin2 appears to more effectively induce autophagy compared to RAD – alone or combined with MLN. We observed a reduction of autophagy occurring in Torin2 and MLN combined compared to Torin2 alone using ImageStream X flow cytometry. Our results attest that MLN2238 along with Torin2 are both promising drugs. However, additional studies are required to thoroughly evaluate these observations. (This work was supported by Cincinnati Children’s Hospital Medical Center 2012 Summer Undergraduate Research Program (SURF)).
POSTER #2

EFFECT OF Diallyl Trisulfide (DATS) ON MIEN1 MEDIATED PROSTATE CANCER PROGRESSION.

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MIEN1, a novel tumor biomarker located in the chromosome 17q12 amplicon, is highly expressed in prostate cancer cell lines and tumors, compared to minimal expression in normal prostate cells and tissues. MIEN1 expression has been reported to positively correlate with grade and stage of prostate cancer progression. It is known that MIEN1 is involved in the migration and invasion of prostate cancer through isoprenylation, a post translational modification. MIEN1 contains a CVIL motif and is modified by the enzyme geranylgeranyltransferase-I (GGTase-I) facilitating its association with the inner leaflet of the plasma membrane. Prenylated MIEN1 induces filopodia formation and promotes cell migration. Previous studies have shown that the prenylation of MIEN1 can be inhibited by GGTL-DU40, a chemical inhibitor of GGTase-I. Diallyl trisulfide (DATS), a garlic-derived chemopreventive organosulfur compound, has been shown to induce disruptions of microtubule formation in human colon cancer cells by reacting with the sulfhydryl groups in prenylated proteins resulting in mitotic arrest and apoptosis. This led to the overall hypothesis that DATS reduces cell migration through MIEN1 in prostate cancer. The purpose of this study is to compare the effects of DATS on cell proliferation and cell migration in DU145 prostate cancer cells with downregulation of MIEN1. By using different methods like MTT cell proliferation assay and scratch assays for cell migration, the effects of these inhibitors were studied. We saw that DATS inhibits the growth of DU145 cells which has high expression of MIEN1 as compared to PWR1E cells (normal prostate cells) which has low expression of MIEN1. In conclusion, our data show that DATS is an effective inhibitor of cell proliferation and migration through its effect on MIEN1 prenylated protein. (This work was supported by U.S. Department of Education McNair grant P217A030039)
SWEET TASTANTS INFLUENCE INSULIN SYNTHESIS IN B CELLS IN ISLETS OF LANKERHANS

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Longitudinal studies of humans have linked consumption of artificial sweeteners with the development of obesity, type 2 diabetes, and certain metabolic syndromes. Prototypic sweet taste receptors are present on taste cells where they function in sense carbohydrate-rich foods. Theses receptor are also present on beta (β) cells in islets of Langerhans, however, their function is unknown. Artificial sweeteners separate carbohydrate sensing from simple hedonic stimulation. Activation of taste receptors without nutrient content should allow us to separate nutrient containing effects from receptor activation alone. A total of nine 3-month old male C/57B mice were divided into two experimental group and one control group and each group received unique drinking water interventions that lasted eight weeks. Group one, filtered water; Group two, 100mM of nutrient-containing sucrose; and group three 0.17mM of non-nutrient containing sucralose). At the end of eight weeks, the mice were given an oral doses of 2 mg/kg body weight glucose after an overnight fast. Several time interval following glucoses dosing, blood was collected for measuring glucose and insulin levels and pancreas was remove for cryostat-frozen sections followed by immuno staining for insulin and glucagons in in islets of the pancreas. We found that mice drinking sucrose and sucralose had lower fasting blood glucose and lower circulating blood glucose after a single the oral glucose. However, we found that insulin content of β cells was increased by both sucrose and sucralose. Our results suggest that activation of sweet receptors on β cells increases insulin content without the requirement of nutrients.
HYPERTENSION IS ASSOCIATED WITH AN INCREASE IN THE NUMBER OF CATECHOLAMINERGIC NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT

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The purpose of this study is to determine if hypertension (high blood pressure) alters the number of catecholaminergic neurons in the Nucleus of the Solitary Tract (NTS). **Methods:** Four adult spontaneously hypertensive rats (SHR) and 2 adult normotensive Wistar-Kyoto rats (WKY) were anesthetized, transcardially perfused with fixative and the brains removed and the hindbrain cut into 40μm thick sections on a cryostat. Every third section containing the NTS was treated with a monoclonal antibody against tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of catecholamines. Alternate sections were treated with an antibody to NeuN which is a neuron-specific protein. All sections were treated with a biotinylated secondary IgG and TH and NeuN were visualized after treatment with DAB. Sections were mounted on glass slides, cover slipped and examined on a confocal microscope. The number of TH-immunoreactive and NeuN-immunoreactive neurons was counted using NIH Image software and the average number of labeled neurons per section calculated. Differences were analyzed using Students t-test and significance accepted at p<.05. **Results:** The arterial blood pressure was significantly greater in SHR (148±10mmHg) compared to WKY (98±4mmHg). The number of TH-immunoreactive neurons was greater in SHR (56±5 cells/section) than WKY (36±8 cells/section). In contrast, the number of NeuN-immunoreactive neurons was not significantly different in SHR (192±30 cells/section) compared to WKY (200±30 cells/section). **Conclusion:** Our results indicate that the total number of NTS neurons, as indicated by NeuN immunoreactivity, is not altered in hypertension. However, there is a significant increase in catecholaminergic neurons in the NTS in hypertensive rats. This demonstrates that the number of catecholaminergic neurons in the NTS is altered in hypertension. Future studies will be necessary to distinguish whether this alteration contributes to the hypertension or represents an adaptation to the hypertension. (Funded by the U.S Department of Education, McNair grant P217A030039 to Dr. Kaman)
The shiga-like toxins (Stx1 and Stx2) produced by shigatoxigenic (STEC) Escherichia coli are important virulence factors for enterohemorrhagic E. coli (EHEC) strains, a STEC subset associated with significant human illnesses, including bloody diarrhea and hemolytic uremic syndrome. E. coli O157:H7 and six non-O157 strains (O26, O111, O103, O121, O45, and O145) are notorious among food safety investigators by virtue of their association with gastroenteritis outbreaks and national food product recalls that typically affect meat and dairy products, raw vegetables, and apple cider. E. coli O157:H7 alone accounts for 73,000 cases of food-borne diseases in the US. A study of 859 American dairy farms found that 4.2% of bulk tank milk samples contained bacteria that carried the stx1 and/or stx2 genes. Although E. coli O157:H7 added to apple cider and treated for 14 s at 68°C showed a 5.0 log reduction in viable cells, it has been reported that Stx2 can survive batch (30 min at 62°C) or high temperature-short time pasteurization (HTST) conditions (15 s at 72°C) accepted for dairy products. A kinetic study was performed to determine the rate of Stx2 inactivation in whole milk treated at different temperatures. Stx2 showed no measurable loss in activity when treated at 55 or 65°C for up to 60 min. The rate of thermal inactivation of Stx2 in milk at 75°C exhibited a $k = 0.14 \pm 0.01$ min$^{-1}$ and a $t_{\frac{1}{2}} = 5.0 \pm 0.1$ min. Thermal inactivation of Stx2 cytotoxic activity at 85 or 95°C occurred too rapidly to measure. These results indicate that some batch and HTST pasteurization conditions used for dairy products are inadequate for complete inactivation of Stx2 cytotoxic activity.
SOY ISOFLAVONE PHYTOESTROGEN, GENISTEIN, DOES NOT PROTECT AGAINST ALCOHOL INDUCED OSTEOPOROSIS IN MALE MICE

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Alcohol abuse acts as a risk factor for osteoporosis by increasing osteoclast activity and decreasing osteoblast activity in bone. Soy proteins are suggested to have beneficial effects on bone in men and women, particularly the isoflavone phytoestrogen, genistein. In this study, male mice were pair fed (PF) a control diet, an EtOH diet or EtOH diet supplemented with genistein (250 mg/kg) for 8 weeks. Ex vivo microCT analyses of formalin fixed tibias from each group revealed a significant decrease in trabecular bone in the EtOH group in comparison with the pair-fed control in regards to BV/TV, Tb.N, and Tb.Sp, p<0.05. No protective effect by genistein was seen in the EtOH+genistein group compared to the EtOH group in BV/TV, Tb.N, and Tb.Sp. Interestingly, there was an increase in Tb.Th in the PF+genistein group compared to the PF, suggesting genistein affects bone remodeling. In ex vivo bone marrow cultures, EtOH exposure decreased the number of alkaline phosphatase stained-pre-osteoblasts compared to PF controls. In contrast, exposure to EtOH+genistein increased pre-osteoblast numbers compared to the EtOH-treated group, p<0.05. These findings suggest that genistein has a partial protective effect since the ALP cell culture displayed an increase in osteoblastogenesis with the addition of genestein. In conclusion, genistein does not protect against ethanol induced bone loss despite the increase in osteoblastogenesis because it does not decrease osteoclast activity which continues to outpace osteoblast activity.
ROBOTIC MODELING AND THE ENHANCE OF THE STEM SUBJECTS

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In order to motivate, advance, and enhance learning of STEM concepts and methods by today’s students, applications of new technology in teaching and learning are urgently needed. LEGO® NXT Mindstorms Robotics and LEGO® Green City Resources is a novel learning supports tool that can inspire at-risk, underperforming, minority students, in grade 7th through 12th to excel in STEM (Science, Technology, Engineering, and Mathematics) related subjects. LEGO® NXT Mindstorms Robotics has the potential to enhance learning and critical thinking skills in chemistry, biology, trigonometry, technology, toxicology, circuits, and engineering. One of the goals of Scientific Research (SR1), a non-profit organization based in Jackson Mississippi, is to increase the number of high-risk students getting into college. To achieve this objective, 12 students and 30 high school science teachers were recruited into a six-week robotic modeling program centered on enhancing learning of STEM bases concepts. The program guides the students with hands on building of and programming of multiple functional robots (what is also known as robotic modeling). The process of building strategies and programming with motion, sensory, and mathematical calculation material, increased their critical thinking skills dramatically throughout the 6-week program. This experience has motivated up 40 to 50 percent of the students to pursue further education and a desire to major in a STEM discipline. The outcome from the Middle and high school students who were taught by the SR1’s mentoring team over the six week program were more likely to say that they would pursue further education after high school, and have gained interest in STEM majors. Thus, providing this type of workshops and tutorial services using robotic modeling, will benefit not only students but also the teachers of STEM subjects. (This program was supported by Scientific Research (SR1), Jackson, MS)
POSTER #8

COMPARISON OF AVAILABLE “FEED STOCK” FOR BIODIESEL PRODUCTION

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Sustainability research is becoming a worldwide concern that has various possible resolutions. In order to carry on sustainability one must consider how these possible resolutions affect the environment and population. Biodiesel fuel is a sustainable alternative fuel used in the place of crude oil or fossil fuels. The major bottleneck in utilizing this sustainable fuel source is “feed stock” used to produce the biodiesel. The purposes of the Southern University Global Sustainability 2012 Summer Initiative was to introduce student to the current “feed stock” material been used by both the USA and China as sources of biodiesel. The program consisted of a two week introduction to sustainability research and a four week visit to China. Analysis of the different types of “feed stocks” been used for biodiesel reveal that the most popular “feed stock” used in American and China is soy. Soy is a highly available vegetable across the nations and can be grown in most farms making it very attractive to industries involved in producing biodiesel. Furthermore, the uses of soy and other “feed stocks” to generate alternative fuel will likely help reducing adverse effects in the environment. Our research suggests that if alternative “feed stocks” can be proved to perform as well or better than petroleum oil bases fuel in a diesel engine; people will have to choose whether they want a cleaner environment or faster performing cars. (this work was supported by NSF-HBCU-ACE grant)
Primary open angle glaucoma (POAG) is a leading cause of blindness affecting 70 million people worldwide. The major risk factor for developing POAG is elevated intraocular pressure (IOP) resulting from increased resistance of aqueous humor outflow through the trabecular meshwork (TM). Bone morphogenic protein-4 (BMP-4) blocks TGF-ß2 induced extracellular matrix (ECM) deposition in TM cells. Follistatin (FST) and gremlin are BMP antagonists that block BMP-4 inhibitory effects. FST is also known for it’s inhibitory effects on activins (Acts). Information is lacking about the function of FST and Acts, in TM cells. In addition, STC-1 may be a key mediator of BMP-4 inhibition of TGF-2 in human TM cells. The purpose of this study was to determine 1) the effect of Act-A on the expression of ECM genes and proteins and 2) if STC-1 protein is present in cultured TM cells. TM cells were cultured and treated with Act-A at a concentration of 50ng/ml for 3, 6, 12, 24, 48, and 72 hrs. QRT-PCR was used to determine Act-A effects on mRNA expression of ECM genes fibronectin (FN), PAI-1, and Collagen 1 in human HTM5 and GTM3 cells. Western immunoblot analysis was used to evaluate Act-A effects on ECM proteins FN, PAI-1, and Collagen 1. TM cells were also cultured with TGF-ß2 (5ng/ml), BMP-4 (10 ng/ml) and/or gremlin (1ug/ml) for 48hrs. Western immunoblot analysis was subsequently used to evaluate protein expression for STC-1 in TM cells. Act-A increased mRNA expression and protein levels of FN, PAI-1, and Collagen 1 in human cultured TM cells. Protein expression of STC-1 was down-regulated by TGF-ß2 and up-regulated by BMP-4 in TM cells. This is the first report of Act-A induction of FN, PAI-1, and Collagen 1 in human cultured TM cells. We have also identified STC-1, a potential mediator in BMP4 attenuation of TGF-ß2 induced ECM deposition, in human cultured TM cells. The involvement of Act-A in the increase of ECM proteins may identify another key mediator in ECM remodeling in glaucoma pathology. The identification of STC-1 in TM cells may be of importance in BMP-4 regulation of TGF-ß2. (This research is funded by U.S. Dept. of Education, McNair: P217A0300 to Dr. Kaman.)
POSTER #10

GENE EXPRESSION CHANGES IN THE VISUAL CENTERS OF THE BRAIN IN BALB/cJ MICE AFTER OPTIC NERVE CRUSH

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Glaucoma is a progressive optic neuropathy characterized by axonal impairment, retinal ganglion cell (RGC) loss, and visual field defects. In the mouse, the majority of RGC axons terminate in the superior colliculus of the brain, and understanding temporal changes in global gene expression patterns post crush will help identify pathways associated with synaptic plasticity and regeneration in glaucoma pathogenesis. The purpose of this study was to evaluate changes in superior colliculus gene expression using an in vivo optic nerve crush (ONC) mouse model that mimics many features of glaucoma axonopathy. Unilateral ONC was performed on 8-10-week-old BALB/cJ eyes using the Nickell’s technique. Superior Colliculus samples (N=5) were harvested at six different time points (0, 3, 7, 14, 21, and 28 days) post crush. Pooled RNA samples (N=5/time point) were run on Affymetrix Mouse Gene array chips. Differentially expressed genes were identified using Partek, and DAVID databases were used for bioinformatics analysis. Temporal expression clusters were statistically identified (p < 0.05) and genes specific to neurogenesis and apoptosis associated cluster temporally graphed. qRT-PCRs were performed to validate altered expression of key dataset genes, including NEUROD4, SNCG, and TCFAP2B. After ONC, gene expression was significantly and temporally altered (p<0.05, fold change of 1.5) in 22 up-regulated gene clusters and 29 down-regulated clusters, based on the three gene ontologies. Early up-regulated gene clusters at 3 and 7 days included regulation of transcription, T-cell mediated immunity, while later time points included neuron projection, neuron differentiation, and sensory eye development. Down-regulated clusters at early time points included immune response, actin filament bundle formation, and regulation of apoptosis. The later time points showed the categories of immune processes as well as cytoskeleton and positive regulation of developmental process. Key genes in the up-regulated dataset included SNCG, and TCFAP2B, while the down-regulated dataset included NEUROD4. qRT-PCR confirmed expression of these genes. Temporal changes seen within key up-regulated clusters are neuronal projection and differentiation at the 21 day time point, while down-regulated clusters are regulation of apoptosis at 3 days and immune system response at later time points. Our findings will be a crucial source of information in the development of therapeutic strategies to prevent the loss of visual function and assist in neuron regeneration after optic nerve axonopathy. (This work was supported by DoD grant, W81XWH-10-2-0003).
POSTER #11

AN EVALUATION OF THE PKM2 INHIBITORS, COMPOUND 3 AND SHIKONIN, IN NEUROBLASTOMA.

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As the field of oncology has expanded, researchers have sought more novel and less toxic chemotherapeutics. One metabolic enzyme found to be upregulated in cancer cells, the M2 isoform of pyruvate kinase (PKM2), has been of particular interest due to its role as the catalyst of the final and rate-determining step of aerobic glycolysis. Two small molecules, compound 3 and shikonin, were recently found to inhibit PKM2 in cultured cancer cell lines. Our study attempts to further test these small molecules for their effectiveness in vitro, using SH-SY5Y neuroblastoma cell line, while also evaluating their toxicity and suggesting a mechanism of inhibition through the use of molecular modeling. The activity of PKM2 in cultured cells was measured using a lactate dehydrogenase coupled assay while standard MTT assay was used to measure toxicity, and tumorigenicity was measured using an anchorage-independent soft agar assay. We observed that both compound 3 and shikonin inhibited PKM2 activity although results from compound 3 were non-significant. Both compound 3 and shikonin showed significant inhibition of tumorigenicity, but further evaluation of the molecular structures of the small molecules lead us to not believe that tumorigenicity was inhibited but rather that the cancer cells were simply not able to support their energy expenditure with PKM2 inhibited and thus died before division. Our results suggest that both compound 3 and shikonin inhibit PKM2 by mimicking the sites of hydrogen bonding on PKM2’s natural activator for tetramerization, fructose-1,6-bisphosphate. Shikonin is suggested to be the most effective inhibitor because it mimics more sites than compound 3. (Support: Biomedical Research Foundation of Northwest Louisiana)
Bisphenol A (BPA) is a colorless organic solid that is used as a monomer in the production of polycarbonate plastics and epoxy resins, and some applications include the manufacture of food and beverage containers and liners. BPA has low affinity for estrogen receptors (ERs) and high doses of BPA induce ER-mediated estrogenic effects. BPA can also bind to the ER-related receptor (ERR) γ and to the G-protein-coupled-ER (GPER) with higher affinity than to ERs. Several studies have shown effects of BPA in the rat prostate below the lowest observed adverse effect level (LOAEL) for oral exposure established by the U.S. Environmental Protection Agency [50 mg/kg body weight (bw)/day], but the molecular mechanisms mediating these changes remain unknown. We assessed if the expression level of genes coding for ERs, ERRs, GPER, and DNA methyltransferases were changed in the prostate of rats exposed orally to BPA from gestation day 6 until postnatal day 4. There were 13 different dose groups, including two negative controls (vehicle and naïve), nine BPA dose groups (2.5, 8, 25, 80, 260, 840, 2700, 100,000, and 300,000 μg/kg bw/day), and two ethinyl estradiol (EE2) reference estrogen controls (0.5 and 5.0 μg/kg bw/day). Gene expression was assessed in ten samples per dose group by real time quantitative RT-PCR. The results indicate that the tested doses of BPA and EE2, under our experimental conditions, do not induce changes in the expression of the genes that were analyzed. Future studies will assess gene expression at a whole-genome level. Supported by FDA IAG 224-12-0003 /NIH IAG AES12013 and by ORISE.
POSTER #13

GENE EXPRESSION AND DNA METHYLATION STATUS IN THE PROSTATE OF RATS DOSED ORALLY WITH BISPHENOL A FROM GESTATION DAY 6 UNTIL POSTNATAL DAY 90

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Bisphenol A (BPA) is a high-volume industrial chemical used in the manufacture of polycarbonate plastic food and beverage containers and epoxy resin food can liners. A report by the National Toxicology Program indicated some concern about the potential of BPA to cause developmental toxicity in fetuses, infants, and children (effects on brain, behavior, and prostate gland), and called for more studies to address uncertainties. One critical question regarding the toxicity of BPA is its mode of action at low levels. At high concentrations, BPA induces estrogenic effects through the estrogen receptor (ER) binding. BPA can also bind to ER-related receptor (ERR) γ and to the G-protein-coupled-ER (GPER). BPA could also act through epigenetic mechanisms, such as modulation of DNA methylation. In the current study, NCTR Sprague-Dawley rats were dosed from gestation day 6 until parturition by oral gavage, and their pups were directly dosed by oral gavage from postnatal (PND) 1 to PND 90. The dose groups included naïve and vehicle controls, BPA [2.5-300,000 μg/kg body weight (bw)/day], and reference estrogen controls (ethinyl estradiol EE₂, 0.5 and 5.0 μg/kg bw/day). The expression level of genes coding for ERs, ERRs, GPER, and DNA methyltransferases was analyzed by real time RT-PCR in the whole prostate. Global DNA methylation was analyzed by a cytosine extension method and the methylation status of Hpcal1 gene was assessed by methylation-sensitive PCR. No BPA- or EE₂-dependent effects were detected in the endpoints analyzed versus vehicle or naïve controls. Since our results differ from reported work from other laboratories, this work points out that differences in the conduct of the study, such as background levels of estrogenic compounds, route/time of exposure, rat strain, or use of whole prostate versus dissected prostatic lobes, may impact the outcome of the study. Supported by FDA IAG 224-12-0003 /NIH IAG AES12013.
FLIPT1 (SLC22A15) MEDIATES THE UPTAKE OF THE ANTICANCER ADENOSINE ANALOGUE, F-ara-A

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Nucleoside analogues (NA) represent a clinically important class of anticancer and antiviral agents. Their use is limited by primary or acquired drug resistance, which may be mediated by loss of influx transporters required for cellular entry into tumors. FLIPT1 (SLC22A15), which has been identified as an organic ion transporter, is abundantly expressed in bone marrow, implicating a potential role for this transporter in the therapeutic actions of NA. In this study, we report a novel function of FLIPT1 as an efficient transporter for adenine nucleosides, nucleotides and analogues. In particular, FLIPT1 overexpression significantly increased the intracellular accumulation of F-ara-A (9-β-D-arabinosyl-2-fluoroadenine), the metabolically active form of fludarabine, which is the most commonly used purine analogue in the therapy of chronic lymphocytic leukemia (CLL). In stably transfected HEK293 cells overexpressing FLIPT1, F-ara-A uptake was saturable with a $K_m$ of 36 ± 11 μM and a $V_{max}$ of 2450 ± 190 pmol/min/mg protein. Additionally, FLIPT1 mRNA levels were significantly correlated with in vitro cytotoxicity of F-ara-A in cell lines transfected with shRNA probes to the FLIPT1 mRNA transcript. The present results suggest that FLIPT1 transport may play an important role in the intracellular accumulation and cytotoxicity of F-ara-A, which provides new insights regarding the transport systems implicated in the anticancer activity as well as toxicity of fludarabine. (Support: National Institutes of Health Grants)
INTEGRATING LOCAL COMMUNITIES IN THE HEALTH RISK ASSESSMENT PROCESS FOLLOWING THE DEEPWATER HORIZON OIL SPILL—A FOCUS ON VIETNAMESE-AMERICANS.

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Vietnamese-American populations in southeast Louisiana consist largely of commercial and subsistence fisherfolk and represent one of the highest seafood consuming groups in the Gulf South. We collected targeted survey data from a Vietnamese-American fishing community in Orleans Parish Louisiana, to determine shrimp consumption rates, body weights, ages, and genders in order to conduct deterministic and probabilistic health risk assessments tailored to this unique population. Cancer risk estimates and levels of concern (LOCs) for several polycyclic aromatic hydrocarbons were determined using GC/MS in SIM mode on a sample of white shrimp collected from the Gulf with shrimpers from this community. Our approach also developed health risk assessments using LOCs, oral slope factors, and risk levels used by the Food and Drug Administration and the Natural Resources Defense Council. Our results demonstrate the need to include key populations in the risk assessment process and measure risk model parameters in such populations rather than rely solely on generic exposure assumptions.
Inactivation Manganese superoxide dismutase (MnSOD) leads to excessive generation of superoxide in mitochondria. Recently, our laboratory generated and characterized a novel kidney-specific MnSOD KO mouse model that exhibited low expression and activity of MnSOD in distal nephrons, displayed increased oxidant production, but surprisingly showed no alteration in renal function. This led us to hypothesize that the oxidative stress as a result of MnSOD knockdown altered mitochondrial function that stimulated compensatory survival mechanisms in renal cells. Using High Resolution Respirometry and fresh mouse kidney biopsies to assess mitochondrial function, MnSOD KO mice exhibited a defect in mitochondrial complex II+III respiration. Moreover, blue native PAGE showed increased mitochondrial respiratory chain complexes in the KO mice. In addition, mitochondrial protein/mass as well as autophagosome formation and DNA replication/repair was increased in MnSOD KO kidney. These findings suggest that mitochondrial superoxide as a result of MnSOD knockdown leads to mitochondrial dysfunction but also triggers multiple coordinated cell survival signals including mitochondrial biogenesis and autophagy, which could protect the kidney against the chronic exposure to oxidative stress. (Support: NIH Grant 1RO1DK0789361)
EVALUATION OF PERCHLORATE INTAKE ON MATERNAL AND FETAL SERUM T₄ LEVELS USING A BBDR MODEL

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Perchlorate (ClO₄⁻) is both a naturally occurring and man-made chemical widely distributed in the environment with low levels detected in food and drinking water. Disturbances in the maternal hypothalamic-pituitary-thyroid (HPT) axis leading to hypothyroxinemia and hypothyroidism have been shown to cause negative effects on the neurodevelopment of the fetus. Downstream perturbations of maternal and fetal HPT axes following ClO₄⁻ competitive inhibition of sodium iodide symporter-mediated thyroidal iodide (I⁻) uptake have not been evaluated quantitatively. In order to quantitate effects of dietary I⁻ intake, ClO₄⁻ exposure and their interactions on maternal and fetal HPT axis, a biologically based dose response (BBDR) model for HPT axes in the pregnant woman and fetus was developed. The BBDR model includes sub-models for I⁻, ClO₄⁻, thyroxine (T₄) and triiodothyronine (T₃). The model was successfully calibrated for euthyroid, marginal iodide deficiency and ClO₄⁻ exposure. Serum thyroid hormone changes were predicted for dietary I⁻ intake ranging from 75 to 250 μg/day and for ClO₄⁻ exposures of 0.01 to 1000 μg/kg/day. Model simulations suggest that ClO₄⁻ at environmentally relevant ranges of exposure (~0.1 μg/kg/day) does not result in significant decreases in maternal and fetal free serum T₄ concentrations for maternal I⁻ intake of 75 to 250 μg/day. For a daily I⁻ intake of 200 μg/day, the daily dose of ClO₄⁻ required to reduce serum free T₄ (fT₄) levels from representative euthyroid region to a hypothyroxinemic state was estimated to be about 50-fold greater (32 μg/kg/day) than the current reference dose (RfD, 0.7 μg/kg/day). As I⁻ intakes were lowered (150, 100 and 75 μg/day), ClO₄⁻ doses required for similar reductions in fT₄ levels were reduced to 28, 16, and 4 μg/kg/day, respectively. This BBDR-HPT axis model for pregnancy provides a novel tool for public health assessments for endocrine active chemicals found in food and the environment. Funded by the Air Force Center for Engineering & the Environment through 711 HPW/RHDJ, WPAFB, OH.
INHIBITION OF ADIPOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS BY TiO2 NANOPARTICLES

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Nanotechnology has resulted in the creation of many new materials and devices with a vast range of applications. TiO2 has historically been used as a pigment with many applications, and nanoscale TiO2 is additionally appearing in human consumer products, raising safety issues of human health and environmental concerns. Stem cells are proposed to be attractive tools for toxicity testing because of their sensitivity to external stimuli during differentiation. The physical and chemical properties of TiO2 nanoparticles were characterized using transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), Brunauer-Emmett-Teller (BET), Raman spectroscopy, X-ray fluorescence spectroscopy (XRF) and other instruments. We examined the impact of TiO2, and other nanomaterials, on cytotoxicity and differentiation of human mesenchymal stem cells (hMSC). TiO2 nanoparticles induced cytotoxicity in a concentration-dependent manner in hMSCs. Additionally, the differentiation of hMSCs to adipocytes, determined using imaging and Oil Red O staining, was inhibited in a concentration-dependent manner. Moreover, uptake of the TiO2 nanoparticles by hMSCs was confirmed using TEM, suggesting a possible endocytosis pathway. The mRNA expression for the adipogenic markers adiponectin, aP2, and LPL were significantly reduced to 61%, 53%, and 61% of control levels following exposure to TiO2 nanoparticles. Furthermore, incubation of the TiO2 with the mesenchymal stem cell lysate resulted in the identification of 208 proteins associated with the nanoparticles using proteomic and mass spectrometry analyses. These results indicate (i) the interaction and impact of nanoparticles with stems cells is selective (other nanoparticles did not induce this effect), (ii) TiO2 nanoparticles inhibited differentiation of mesenchymal stem cells, and (iii) further work is needed to elucidate the mechanism of action of TiO2 in this cell population.
Ketamine is a dissociative anesthetic that is frequently used for the induction and maintenance of general anesthesia in children. It has been reported that blockade of NMDA receptors by ketamine may cause neurotoxicity in neonatal rats when given over a 12 hour period during the brain growth spurt. Noninvasive, quantitative imaging of rodent brains may allow for the detection of functional, morphological and metabolic alterations induced by ketamine. Since it is known that the levels of peripheral benzodiazepine receptors (PBRs) increase in areas of neuronal injury following exposure to neurotoxicants, PBRs are widely recognized as important targets for imaging using positron emission tomography (PET). In this study, the effect of ketamine on the uptake and retention of [18F]-FEPPA in the brains of rats and the potential protective effect of minocycline, an anti-inflammatory agent, on anesthetic-induced neuronal cell death were investigated using microPET/CT imaging. On PND 7, rat pups in the experimental group were exposed to 6 injections of ketamine (20 mg/kg at 2 h intervals) with or without minocycline (45mg/kg i.p.,30 minutes prior and 4 hours after exposure) control pups received 6 injections of saline. On PNDs 14, 21, 28 and 35, [18F]-FEPPA (18.5 MBq) was injected into the tail vein of treated and control rats and microPET/CT images were obtained over the next 90 minutes. Radiolabeled tracer accumulation in regions of interest (ROIs) in the frontal cortex was converted into Standard Uptake Values (SUVs). In PND 14 and 21 rats the uptake of [18F]-FEPPA was significantly increased and the duration of tracer wash-out was prolonged in ketamine-treated rats. The increased uptake of the tracer was attenuated by the co-administration of minocycline. On PND 28 and PND 35, however, no significant difference in radiotracer uptake in the frontal cortex was found. This preliminary study demonstrates that microPET imaging is capable of distinguishing differences in retention of [18F]-FEPPA in the brains of rodents and suggests that this approach may provide a minimally invasive biomarker of pathogenic process associated with neurotoxicity induced by ketamine. Minocycline effectively blocks the neuronal injury caused by ketamine anesthesia in the developing rat brain. (Supported by NCTR/FDA; E-7285)
MUTAGENIC POTENCY OF FIVE CHEMICALS CONTAINED IN CIGARETTE SMOKE

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Cigarette smoke contains more than 8,000 different chemical compounds and 81 of them have been classified as carcinogens by the IARC. Our previous study found that cigarette smoke condensates from 11 cigarette brands resulted in dose-dependent increases of cytotoxicity and mutagenicity and that the samples varied in their cytotoxic and mutagenic potency. In the present study, we evaluated the cytotoxic and mutagenic potency of five chemicals, 4-ABP, BP, CdCl₂, NNK, and MeIQ selected to represent different chemical classes present in cigarette smoke. L5178Y/Tk⁺/- 3.7.2C mouse lymphoma cells were treated with various concentrations (0.2 μM - 2 mM) of the five chemicals for 4 hours. In the absence of metabolic activation, only CdCl₂ showed a positive response. In the presence of S9, CdCl₂ showed moderate mutagenicity (364×10⁻⁶) while the other four chemicals induced a substantially higher mutant frequency (MF); 1260-1892×10⁻⁶ at the highest acceptable test concentration. The order of mutagenic potencies calculated using the slope of the linear regression of the dose-response curve were: BP, 141.5; CdCl₂, 65.0; MeIQ, 13.6; 4-ABP 9.5; and NNK 0.4×10⁻⁶ MF per μM. Of the five chemicals, BP was the most potent mutagen and NNK was the least mutagenic. The contribution of each of these chemicals to the overall mutagenicity of cigarette smoke will vary based on the tobacco blend and chemical composition of the smoke. For instance, the concentration of NNK is generally much higher in smoke than 4-ABP so while NNK is a less potent mutagen, it may induce more mutants than 4-ABP for a given quantity of smoke consumed. These experiments are designed to inform analyses of whole cigarette smoke which is a complex chemical mixture.
IN VITRO GENOTOXIC EFFECTS OF GINKGO BILOBA EXTRACT AND ONE OF ITS MAJOR COMPONENTS, QUERCETIN

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Ginkgo biloba (ginkgo), one of the world’s oldest living tree species, has been used for many years for a variety of medicinal purposes. The NTP 2-year bioassays on ginkgo extract found an increased incidence of liver cancer in mice and thyroid gland cancer in both mice and rats. The mouse lymphoma assay (MLA) was used to evaluate the in vitro mutagenicity of ginkgo extract and one of its major constituents, quercetin. L5178Y/Tk+/-3.7.2C mouse lymphoma cells were treated with different concentrations of ginkgo extract (0.2-1.2 mg/ml) and quercetin (3-30 μg/ml) in the absence of metabolic activation (S9). Both ginkgo extract and quercetin significantly increased the mutant frequency in the MLA. Loss of heterozygosity (LOH) analysis for mutants induced by quercetin was also examined at five microsatellite loci spanning the entire mouse chromosome 11. The results indicated that large deletion on the mouse chromosome 11 from the quercetin treatment was significantly different from that of the negative control. Additionally, a neutral comet assay conducted in the mouse lymphoma cells with different doses of quercetin demonstrated a significant, dose-dependent increase in the DNA double-strand breaks (DSBs). Western blot analysis showed that quercetin increased the phosphorylation of ATM, and consequently increased expression of γ-H2AX, phosphorylated Chk1 and Chk2 in the cells. These results suggest that ginkgo extract and one of its major constituents, quercetin, are genotoxic in the mouse lymphoma cells.
COPPER NANOPARTICLES OR IONIC COPPER (II) CAUSES NEUROTOXICITY AND CARDIOTOXICITY IN ZEBRAFISH EMBRYOS


Div. of Neurotox, NCTR, US FDA

Copper oxide nanoparticles (Cu-NPs) are frequently used in industrial applications for medical devices, paints, fabrics and antimicrobials. In this study, we investigated the toxicity of Cu-NPs and ionic copper(II) in wild-type (WT) zebrafish hb9-GFP transgenic zebrafish (Danio rerio, AB-strain) embryos by comparing bare Cu-NPs to the mass equivalent ionic from of copper(II) (CuCl₂). Both Cu-NPs and CuCl₂ were lethal to zebrafish embryos at 20 ug/ml (within 24-hrs) and 10 ug/ml (within 48-hrs), with CuCl₂ being more toxic at equivalent mass concentrations. Similarly, the heart rate was significantly reduced following exposure to either Cu-NPs or CuCl₂ in a concentration-time dependent manner. The embryo permeability studies showed that exposure to either Cu-NPs or CuCl₂ (5 ug/ml for 24-hrs) significantly increased absorption of the fluorescent tracer 6-coumarin (6CM). Embryos treated with either Cu-NPs or CuCl₂ (2.5 ug/ml for 48-hrs) showed a significant reduction (nearly 2-fold) of spinal motor neurons. These results indicate that both CuCl₂ and Cu-NPs can be toxic to zebrafish embryos causing significant neurotoxicity and cardiotoxicity at exposure levels that do not cause lethality.
The dissolution potential of citrate capped silver nanoparticles (AgNPs) in laboratory test media and in the environment is critical for determining toxicity. In the present study, the ion-release kinetics from citrate capped 20, 50, and 80 nm AgNPs in dilutions of an environmentally relevant freshwater (30μS/cm and 150μS/cm reconstituted water) was examined and related to the associated impact on an aquatic organism (*Daphnia magna*). Diluted suspensions of nanoparticles were placed on a multi-tube vortexer and orbital shaker for 0, 1, 2, 3 and 7 days. The acute toxicity of the AgNPs suspensions was then assessed with *D. magna* at 0 and 7 days post interaction between the particles and test media. An increase in hydrodynamic diameter measured by dynamic light scattering and field flow fractionation over time was observed at a relatively higher specific conductivity of 150μS/cm in 20nm particles (3.3 fold increase) and only a small increase in 50 and 80nm particles (1.4 and 1.2 fold increase, respectively). At a lower conductivity of 30μS/cm; a 1.7, 1.0 and 1.2 fold increase was observed in 20, 50 and 80nm, respectively. Results showed that although the total concentration of silver in solution decreased with time, there was a consistent spike in dissolved concentration after 2-3 days interaction, followed by a steady decrease in dissolved silver in 150μS/cm and 30μS/cm medium. This suggests that the concentration of dissolved silver in environmentally relevant ionic strength media increases over time after the introduction of citrate capped AgNPs which may have implications on aquatic organisms. When exposed *D.magna* was exposed to 150μS/cm and 30μS/cm test media, 30μS/cm test media induced more toxicity than 150μS/cm test media. Toxicity increased with longer nAg interaction time with smaller particles inducing more toxicity than larger particles.
POSTER #24

ROS/REDOX SIGNALING REGULATES BONE TURNOVER IN AN AGE-SPECIFIC MANNER IN FEMALE MICE.

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In bone, oxidant signaling through NADPH oxidase (NOX)-derived reactive oxygen species (ROS) superoxide and/or hydrogen peroxide appears to be an important stimulus for osteoclast differentiation and activity. We have shown previously that alcohol abuse (EtOH), generates excess Nox-dependent ROS in osteoblasts, which functions to inhibit bone formation through impairment of Wnt signaling, and increases osteoclastogenesis by stimulating RANKL-RANK signaling. These effects can be blocked by the dietary antioxidant N-acetylcysteine and the pan-Nox inhibitor DPI. In the current study we utilized a well-described transgenic C57Bl/6J mouse strain which over-expresses human catalase (TgCAT) in all tissues to see if limiting excess hydrogen peroxide production in bone would protect against EtOH-mediated bone loss. Six wk old, wild-type (WT) and TgCAT female mice were then given rodent chow ad libitum, or pair-fed (PF) liquid diets with 0% or 30% EtOH calories for 8 weeks. Interestingly, at 6 wks, microCT analysis of chow fed TgCAT mice revealed an osteopetrotic phenotype, increased BV/TV, Tb.N. and Tb.Sp., in trabecular bone when compared to aged-matched WT mice, p<0.05. However, EtOH-feeding decreased bone strength and stiffness in both WT and TgCAT mice. MicroCT analysis of chow fed WT and TgCAT mice at 14 wks revealed an osteoporotic phenotype associated with TgCAT over expression later in development. These data suggest that ROS/redox signaling regulates bone turnover in an age-dependent fashion. (Support: R01 AA018282 (M.J.R.).)
VITAMIN D SUPPLEMENTATION PREVENTS HYPOCALCEMIA AND CORTICAL BONE LOSS ASSOCIATED WITH CHRONIC ALCOHOL FEEDING IN FEMALE MICE.

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Dietary cholecalciferol supplementation alone or combined with calcium has shown great promise in improving bone health, which has been attributed to endocrine actions involved in calcium regulation and/or paracrine/autocrine actions within bone. In the current study, 6 wk old, female C57BL/6J mice were pair-fed (PF) LieberDeCarli liquid diets containing 0% or 30% EtOH supplemented with 400 IU (EtOH/400) or 2000 IU (EtOH/2000) of cholecalciferol for 40 d. In the EtOH/400 group, chronic EtOH feeding resulted in decreased bone strength and stiffness (p<0.05), reductions in trabecular BV/TV and cortical volumetric BMD (p<0.05), and increased biochemical markers of bone resorption. The levels of circulating 1,25(OH)2D3 and ionized calcium were significantly decreased in the serum (p<0.05), and apoptosis was increased the bone marrow cells when compared to PF controls. In contrast, increasing daily cholecalciferol intake from 400 to 2000 IU/kg, completely prevented the cortical bone loss by reducing EtOH-mediated increases in bone resorption and protected against EtOH-mediated hypocalcaemia. In cultured cells, pre-treatment of 1,25(OH)2D3 in EtOH-treated ST2 cells protected against increased caspase-3 activity. In the EtOH/2000 mice, circulating 1,25(OH)2D3 was significantly lower compared to mice receiving EtOH alone, suggesting increased sensitivity to feedback control of vitamin D metabolism in the kidney. These data suggest that daily dietary intake of cholecalciferol of 2000 IU may protect against bone toxicity associated with chronic alcohol abuse in younger women, thus reducing the increased risk of osteoporosis and fracture that comes with age. (Support: R01 AA18282 (M.J.R.)).
SILENCE OF SOX2 INDUCES APOPTOSIS THROUGH BOTH MITOCHONDRIA AND DEATH RECEPTOR SIGNAL PATHWAY BY ACTIVATING THE RAS/MAPK SIGNALS IN HUMAN LUNG CANCER CELLS

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Previous study showed that the stem cell gene-SOX2 plays an important role in the anti-apoptotic property of tumor cells, but the regulatory mechanism of SOX2 in the apoptosis signals is still elusive. In this study we used lentivirus system to deliver the shRNA to human lung cancer cell line-A549 and H460 and found that silencing of SOX2 gene can effectively induce apoptosis. Proteomic array assay and western blotting showed that down-regulation of SOX2 activates both the mitochondria and death receptor signal pathway, which were marked by the overexpression and phosphorylation of p53, overexpression of Bax and Bad, and activation of caspase 3 and 8 in A549 cells with SOX2 silencing. Real-time RT-PCR showed that down-regulation of SOX2 leads to the activation of Ras/MAPK signal pathway, which activated the transcription of two key inducers for apoptosis signals-TNF-α and p53 and also down-regulates the expression of survivin. In vitro and in vivo experiment further showed that this apoptotic effect of SOX2 silencing was compromised by overexpression of survivin. Immunohistochemistry study of human lung tissues showed that the protein expression level of SOX2 is closely correlated with that of survivin. In addition, SOX2 and survivin double positive patients showed worse prognosis, suggesting these two proteins can be used as dual-diagnosis factors to evaluate the clinical progression and prognosis of lung cancer patient. These findings revealed the important mechanism for SOX2 to regulate the apoptosis signals and demonstrated that survivin is one of vital downstream molecules that contribute to the anti-apoptosis property of SOX2.
IN VIVO DEMONSTRATION OF FLUORO-TURQUOISE CONJUGATED GELATIN FOR THE DEMONSTRATION OF ENDOTHELIAL CELLS IN THE BRAINS OF HEALTHY AND KAINIC ACID EXPOSED RATS

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Although a few methods exist to visualize brain vasculature of rodents, even fewer are applicable in all animal models of different diseases. For example, the immunohistochemistry methods are useful but most blood vessels in the paraformaldehyde (PFA) fixed tissues from adult rodent’s brain often stain poorly or not at all and therefore employment of antigen retrieval is necessary to improve immunohistochemical staining. This study describes a new method for the visualization of vasculature lumen and endothelial cells and characterizes their morphology in the normal and excitotoxin, kainic acid, treated rats. Labeling was accomplished by Fluoro- Turquoise (FT), a novel reactive blue fluorochrome, conjugated to gelatin. Strong fluorescence of FT gel was found in the vasculature throughout the brain following intra-cardiac perfusion with FT gel in the normal animals. However in the KA injected animal’s brain (hippocampus, midline and ventral thalamus, piriform cortex) the injured areas, in the vascular lumen is typically faintly stained and constricted. The FT-gel can also be used to visualize endothelial cells in the mouse brain and has tremendous usefulness, as there is no marker available to observe endothelial cells in the mouse brain. Potential advantages over other markers can be attributed to its unique chemical and spectral properties. Specifically, it results in a very bright blue UV excitable stain that does not bleed through when illuminated with other filters. Its brightness at low magnification makes it ideal for multiple immunofluorescent labeling and high volume quantification studies.

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IDENTIFICATION OF GENETIC VARIANTS IN DISEASE RISKS AND DRUG RESPONSES BY NEXT-GENERATION SEQUENCING


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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 phenotypes of (i) triglyceride response to a high fat challenge meal, (ii) blood pressure response to high salt diet, and platelet-aggregation response to (iii) aspirin and (iv) clopidogrel administration were associated with risks of cardiovascular diseases. The responses were 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 phenotypes. 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 a phenotypic trait. Candidate variants associated with specific phenotypes 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. Data analyses for the other three phenotypic traits are ongoing.
POSTER #29

DIGLYCOLIC ACID, THE NEPHROTOXIC METABOLITE OF DIETHYLENE GLYCOL, INHIBITS INTRACELLULAR 14C-SUCCINATE UPTAKE IN HUMAN PROXIMAL TUBULE CELLS IN VITRO

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Diethylene glycol (DEG) is an organic solvent used in antifreeze blends, brake fluid, and the production of various polymers allowing a risk for consumer exposure. Acute renal failure caused by cortical tubular degradation and proximal tubule necrosis is the trademark of DEG poisoning. DEG is ultimately metabolized to 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA) with DGA being the proximate toxicant. DGA has a strong structural resemblance to various Krebs Cycle intermediates, particularly succinate. Human proximal tubule (HPT) cells transport dicarboxylates such as succinate via sodium-dicarboxylate (NaDC) transporters, including NaDC-1 which is primarily found on the apical surface of PT cells. Due to a specificity for succinate, we have hypothesized that the ability of NaDC-1 to transport succinate into HPT cells is inhibited in the presence of DGA. HPT cells were co-incubated for 2 h with a constant concentration of C-14 labeled succinate (50μmol/L; 0.1mCi/mL), along with increasing concentrations of DGA (0-500 mmol/L). Results indicate that increased concentrations of DGA inhibit the intracellular uptake of C-14 labeled succinate by greater than 50%. An IC50 value of 175 mmol/L was determined for intracellular succinate uptake in the presence of increasing DGA concentrations. The high IC50 suggests that DGA-induced cytotoxicity is not a result of succinate starvation, but rather an inhibition of metabolic processes at much lower DGA concentrations.
TRICHLOROETHYLENE INDUCES EPIGENETIC ALTERATIONS IN CD4⁺ T CELLS


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Previous studies have shown that chronic (32-week) exposure to occupationally-relevant concentrations of trichloroethylene (TCE) in the drinking water of female MRL+/+ mice promoted autoimmune hepatitis. This was accompanied by the expansion of CD4⁺ T cells that secreted increased levels of IFN-γ and expressed an activated (CD44↑CD62L↓) phenotype. A recent longitudinal study of TCE-induced autoimmune disease revealed that the alterations in IFN-γ production corresponded to changes in the expression of markers used to assess DNA methylation, namely retrotransposons Iap (Intracisternal A Particle) and MuERV (murine endogenous retrovirus). Also altered by TCE exposure was expression of Dnmt1 (DNA methyltransferase-1), and several genes known to be regulated by DNA methylation, namely Ifng, Il2 and Cdkn1a. In addition, DNA collected from the CD4⁺ T cells of TCE-treated mice showed TCE significantly altered global DNA methylation. Most recently, bisulfite sequencing revealed that DNA methylation of CpG sites associated with the Ifng promoter was significantly, and time-dependently altered by TCE exposure. Thus, for the first time, a toxicant known to promote autoimmune disease has been shown to alter epigenetic processes (DNA methylation) in the cell type that mediates pathology, namely CD4⁺ T cells.

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SIZE DEPENDENT ANTIBACTERIAL EFFECTS OF SILVER NANOPARTICLES (AGNP) ON BACTERIAL SPECIES OF THE GASTROINTESTINAL (G.I.) TRACT.

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The extensive use of AgNP in the food industry may pose a significant health risk to consumers. It is known that AgNPs can be absorbed in the mammalian G.I. tract; however, the effects of AgNP on bacterial species of the G.I. tract are largely unknown. We investigated the bactericidal effects of AgNP on Lactobacillus sp. (G⁺) and Bacteroides sp. (G⁻) isolated from the G.I. tract of Sprague-Dawley rats. Three sizes of AgNPs (10, 75, and 110 nm) at (0.1, 0.5, 1, 2, 5, 10 μg/ml) were incubated with cultures of bacteria (in triplicate), and the bactericidal effects at 60, 120, and 180 min were assessed using colony forming unit (CFU) and adenosine triphosphate (ATP) release assays. The bactericidal activity of AgNP generally increased as particle size decreased for both bacterial species, suggesting that AgNP 10 nm were more potent bactericidal agents than the 75 or 110 nm. Both bacterial species showed a significant decrease in ATP release when exposed to AgNPs at concentrations > 1 μg/ml. At an equivalent number of cells (10³ cells/mL) Bacteriodes sp. were more sensitive than Lactobacillus sp to the bactericidal properties of AgNP, likely reflecting differences in cell wall composition between species. Scanning electron microscopy showed that AgNPs were attached to bacterial cells at the surface, suggesting that protein binding or cell wall distortion by AgNPs might result in cellular toxicity or death. These results provide new insights into the bactericidal properties of AgNPs and how the consumption of AgNPs might disrupt the normal balance of microorganisms in the healthy G.I. tract. Supported by interagency agreement No. 224-12-0003 and AES12013 between the NCTR/FDA and NIEHS/NTP.
DEVELOPMENT OF DOXORUBICIN-INDUCED CHRONIC CARDIOTOXICITY IN THE B6C3F1 MOUSE MODEL

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Serum levels of cardiac troponins (cTn) serve as biomarkers of myocardial injury. However, troponins are released into the serum only after damage to cardiac tissue has occurred. Here, we report development of doxorubicin (DOX)-induced chronic cardiotoxicity in a mouse model to aid in the identification of early biomarkers of cardiac tissue injury. Male B6C3F1 mice were administered intravenous DOX at 3 mg/kg body weight, or an equivalent volume of saline, once a week for 4, 6, 8, 10, 12, and 14 weeks, resulting in cumulative DOX doses of 12, 18, 24, 30, 36, and 42 mg/kg, respectively. Mice were sacrificed a week following the last dose. DOX treatment resulted in declines in red blood cell count, hemoglobin, and hematocrit compared to saline-treated controls after the 2nd weekly dose until the 8th and 9th doses, followed by a modest recovery. Measurements of cTnT level in plasma revealed significant elevations in all DOX-treated mice compared to saline-treated controls, indicating cardiac tissue injury. In addition, microscopic examination of the hearts showed a dose-related increase in the severity of cardiac lesions in mice exposed to 24 mg/kg and higher cumulative DOX doses. At cumulative doses of 30 mg/kg and higher, mice exhibited drug-induced cardiac dysfunction as evidenced by a significant decline in heart rate. Altogether, these findings demonstrate the development of DOX-induced chronic cardiotoxicity in B6C3F1 mice.
BENZO[a]PYRENE DIETARY EXPOSURE EFFECTS ON REPRODUCTIVE SUCCESS AND F1 DEVELOPMENT IN ZEBRAFISH


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Benzo[a]pyrene (BaP) is an environmentally relevant carcinogenic and endocrine disrupting compound that causes multigenerational effects in mammals. We hypothesized that, like in humans, BaP exposure would adversely affect zebrafish reproduction and cause quantifiable pathologies in the F1 generation. Adult zebrafish (2 females x 2 males, N=10 replicate tanks per treatment) were fed 2% body weight/day flake food treated with 0, 11.6, 110, 1086 μg BaP/g flake (equivalent to 0, 0.23, 2.2, and 22 μg BaP/g fish/day) for 22 days. Parental gonad pathology and reproductive success, and F1 survival and morphological abnormalities were measured. The total number of eggs produced and fertilization success was non-significantly reduced in a dose-dependent manner, and parental ovarian atresia was significantly decreased. Mortality was significantly increased in larvae whose parents were exposed to 2.2 and 22 μg BaP/g fish by 48 and 56 hours post-fertilization (hpf), respectively. High dose BaP F1 fish hatched sooner (48 hpf) compared to control (56 hpf). Body and tail shape and swim bladder were negatively impacted after parental exposure to 2.2 and 22 μg BaP/g fish. Based on these results, BaP negatively impacted zebrafish reproduction and F1 development. Supported by NIEHS R21ES019940.
Green tea has a considerable amount of polyphenolic catechins (potent antioxidants) and it has shown to have protective effects on oxidative stress-related diseases. Acetaminophen (APAP) is a widely used analgesic drug that can cause acute oxidative stress and liver injury. The aim of this study was to explore the effects of green tea extract (GTE) on APAP-induced hepatotoxicity using an UPLC/MS-based metabolomic approach. Male B6C3F1 mice were orally administered GTE (500 or 1000 mg/kg) either 3 h prior to 200 mg APAP/kg oral treatment, or for three consecutive days (once daily) followed by 300 mg APAP/kg on the fourth day. Blood and liver tissue samples were collected 24 h after APAP administration for clinical chemistry, histopathological and metabolomic analysis. UPLC/MS-based metabolomics profiling was utilized to examine metabolic changes in liver extracts. The partial least squares discriminant analysis of the LC/MS data showed that groups dosed with APAP alone were the most distinct from controls, while animals dosed with 500 mg GTE/kg followed by APAP treatment were located between these two and those pre-treated with 1000 mg GTE/kg groups were nearest to control group. These results were consistent with histopathological data showing that 3h single or 3d consecutive dosing with 1000 mg GTE/kg prior to APAP exposure significantly alleviated some of the hepatocyte necrosis compared with APAP dosing alone. Similar results were observed for ALT and AST. Dose dependent changes were observed in the energy pathway-related metabolites, including acylcarnitines (intermediates in fatty acid β-oxidation pathway, mitochondrial injury biomarker) and lysophosphatidylcholines, which returned to controls levels after 3h or 3d consecutive dosing GTE prior to APAP treatment compared to APAP treatment alone. These results indicate that GTE treatments prior to APAP dosing could relieve liver injury.
Mutations in the alpha-synuclein gene have been associated with autosomal dominant forms of Parkinson’s disease (PD). Transgenic mice that over-express the human alpha-synuclein gene (primarily the point mutations A53T and A30P), develop neurological impairments similar to those of PD. Previous studies in our laboratory have shown that oxidative-stress-mediated activation of the tyrosine kinase, c-Abl, results in an increase in the phosphorylation of parkin, an important E3 ubiquitin ligase that assists in the clearance of proteins destined for proteasomal degradation. Here, we show that treatment with iron-oxide nanoparticles or methamphetamine results in activation of c-Abl, observed via the measurement of phospho-Abl. Additionally, an over-expression of alpha-synuclein protein in SHSY-5Y neuroblastoma cells was observed after these treatments. A 45% increase in the expression of alpha-synuclein was observed in SHSY-5Y cells treated with iron oxide nanoparticles (10 and 30 nanometers) at a concentration of 10 μg/ml. Similarly, a 55% increase in the expression of alpha-synuclein protein was observed 24 h after exposure to 500 uM methamphetamine. In addition, a significant depletion in dopamine was observed after treatment with either iron oxide nanoparticles or methamphetamine (55% and 65%, respectively), suggesting that both the over-expression of alpha-synuclein and excess dopamine might generate oxidative stress, which is one of the major pathways of c-Abl activation. These results suggest that the over-expression of alpha-synuclein and dopamine depletion after exposure to iron oxide nanoparticles or methamphetamine contribute to oxidative-stress mediated activation of c-Abl, thus initiating events that may lead to dopaminergic neuronal cell death.

Protocol # E0739401
Oxybenzone is a chemical commonly used in sunscreens and cosmetics due to its ability to absorb UV radiation. A report by the U.S. CDC found that 96.8% of tested human urine samples had measurable levels of oxybenzone. Data from in vitro and in vivo assays have shown oxybenzone to have weak estrogenic activity raising the question of the significance of the exposure to oxybenzone and whether oxybenzone or its metabolites act as endocrine disruptors. To determine if maternal exposure influenced embryonic development, time-mated 12-13 week old Harlan Sprague-Dawley rats were given oxybenzone at concentrations of 0; 1000; 3000; 10,000; 25,000 or 50,000 ppm via a low phytoestrogen chow beginning on gestational day (GD) 6 and ending on GD10, GD15, GD20 or postnatal day (PND) 23. Maternal toxicity depicted by decreased weight gain during pregnancy was observed beginning on GD10 for the two highest dose groups and continued through GD20. No differences were observed in the number of implants or live fetuses, or fetal weight on GD20. At PND23, there were no body weight differences between the dose groups of treated maternal animals; however, 10,000 ppm and greater was associated with higher relative liver weights, suggestive of liver toxicity. Oxybenzone decreased postnatal pup weight in the 50,000 ppm group; this decrease was first detected at PND4. Overall, the LOAEL for oxybenzone was determined to be 10,000 ppm for maternal toxicity and 50,000 ppm for pups.
INCREASED MUCUS PRODUCTION AND HISTOPATHOLOGICAL GILL ALTERATIONS AFTER EXPOSURE TO NANOSILVER AND SILVER NITRATE

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Silver nanoparticles are among the most widely used nanomaterials because of their antibacterial and antifungal properties. Despite their extensive use, information is now becoming available on the toxicity and fate of nanosilver formulations within living organisms. Mucus has both increased and decreased the toxicity of different xenobiotics by either concentrating the xenobiotic on the gills and body or encapsulating toxicants to prevent exposure. In order to understand the relationship between mucus and silver exposure, zebrafish (ZF) and fathead minnows (FHM) were exposed for 36 or 96 hr to nominally 20 nm PVP- or citrate-coated silver nanoparticles (PVP-AgNPs; citrate-AgNPs) or silver nitrate (AgNO₃) at 2 nominal concentrations (20 and 200 μg/L) or (2 and 6 μg/L), respectively. After 4 hr, ZF produced significantly more mucus secretion in every treatment than the control fish in a dose-dependent manner as measured by a phenol-sulfuric acid method. FHM gills were paraffin embedded, sectioned and examined for histopathological lesions. The highest AgNO₃ and both citrate-AgNPs concentrations caused significant atrophy in gill mucus goblet cells. Every silver treatment also had a significantly higher histopathological alterations index compared to control. Citrate-AgNPs (20 and 200 μg/L) had the highest incidence of alterations (3.5 and 3.25 times higher than control, respectively). This research is supported by US Army ERDC grant: W912HZ-09-C-0033.
EVALUATING THE EFFECTS OF PENICILLIN TREATMENT ON THE URINE AND PLASMA METABOLOMES OF SPRAGUE-DAWLEY RATS

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The activity of the gut microbiota has been documented as playing important effects on a host’s physiology and pathology. Hence, evaluation of an antibiotic’s effect on the gut microbiota may provide new insights into the drug’s effects on organs and organism physiology. LC/MS- and NMR-based metabolic profiling was employed to evaluate the effects of penicillin (PEN), a well-known antibiotic widely used in the treatment of bacterial infections, on the host’s metabolic phenotype. Male Sprague Dawley rats were randomly divided into groups that were orally administered either with 0.5% methylcellulose vehicle, 100 or 2400 mg PEN/kg body weight once daily for up to 14 consecutive days. Urine, terminal plasma and tissue were collected from groups sacrificed at 6 h, 24 h or 14 days and were subjected to clinical chemistry, histopathology and metabolomics analysis. Open metabolic profiling of urine and plasma was performed by NMR and UPLC/QToF-MS. Time- and dose decreases in some urinary metabolites (compounds) indicated the gut microflora population was suppressed. These compounds were indole-containing metabolites (such as methylidioxyindole sulfate generated from bacterial metabolism of tryptophan), organic acids containing phenyl groups (such as hippuric acid, phenyllactic acid and 3-hydroxyanthranilic acid), and drug-like phase II metabolites (such as cresol sulfate and aminophenol sulfate). Serum bile acids (e.g., cholic acid and cholic acid isomer), whose hydrolysis and hydroxy group dehydrogenation reactions are only carried out by gut microbiota, showed increases at 6 h postdosing, followed by time- and dose-dependent decreases in the treated groups. The decreases in bile acids at 24 h and 14 days postdosing could be related to the suppressed gut microflora population by PEN. In all, these results clearly showed metabolic profiling is a useful tool to better understand the effects of the antibiotic penicillin has on the gut microbiota and the host organism.
EFFECTS OF BENZO[a]PYRENE ON EARLY ZEBRAFISH DEVELOPMENT

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Benzo[a]pyrene (BaP) is a ubiquitous environmental contaminant that is an endocrine disrupting and carcinogenic high molecular weight polycyclic aromatic hydrocarbon. Our previous work found that BaP significantly decreased fish brain aromatase (CYP19b) expression, a key enzyme in steroidogenesis. We hypothesized that BaP deregulates the steroid hormone hypothalamus-pituitary-gonad feedback loop adversely affecting reproductive development and physiology. Zebrafish embryos were exposed to waterborne concentrations of BaP (0, 10, and 50 μg/L) for 96 hours postfertilization (hpf). Fifty μg/L BaP significantly increased mortality compared with the control and 10 μg/L groups at 24, 48, 72, and 96 hpf, whereas mortality was not significantly increased until 96 hpf in the 10 μg/L BaP group. In order to quantitate effects on larval estrogen and testosterone concentrations, larvae were collected at 48, 72, 96, 168 and 504 hpf. Histopathological assessment of gonad maturation was done on paraffin embedded and sectioned fish at 28, 32, 35, and 52 days post fertilization. In a treatment-blinded morphological assessment of larvae at 96 hpf, the high BaP dose significantly decreased the body length, optic vesicle, and swim bladder size while increasing pericardial and abdominal edema compared to control and 10 μg/L treatments. Body and tail shape and fin malformation scoring also indicated a dose-dependent adverse impact of BaP-treatment on development. Results extend previous studies highlighting the adverse impacts on early development of BaP-exposure. (Supported by NIEHS R03 ES018962)
PREVALENCE OF KRAS MUTANT TUMOR SUBPOPULATIONS

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Tumor mutations, such as those in KRAS codon 12, are being used as predictive biomarkers of therapeutic response, in order to select the most effective treatments for individual cancer patients. Undetected KRAS mutant subpopulations have been implicated in acquired resistance to EGFR-targeted therapies, but the prevalence of undetected KRAS mutant subpopulations is currently unknown. To establish the frequency with which KRAS mutations occur as tumor subpopulations, we determined KRAS G12D and G12V mutant fractions (MFs) in normal and tumor tissue samples using the sensitive and quantitative ACB-PCR method. We find many tumors of the colon, lung, pancreas, and thyroid carry KRAS G12D or G12V mutant subpopulations at frequencies higher than those observed in normal tissues. For example, in lung tumors, 10/25 and 12/25 had KRAS G12D and G12V mutant fractions (MFs) above the upper 95% confidence interval (CI) for the corresponding MFs in normal lung, respectively. Of those, 0/10 and 5/12 had a KRAS G12D or G12V MFs ≥ 10\(^{-1}\) (i.e., that detectable by DNA sequencing). In papillary thyroid tumors, KRAS G12V mutations were present above the upper 95% CI for normal thyroid in 8/19 tumors, even though the COSMIC database indicates this mutation occurs in only 0.15% of papillary thyroid tumors. This is consistent with our observation that none of the papillary thyroid tumors had levels of mutation detectable by DNA sequencing. In addition, we found KRAS G12V is significantly, but inversely, correlated with maximum tumor dimension. In large, advanced tumors, hypoxia-induced production of reactive oxygen species may select against KRAS mutant cells, thereby explaining their frequent occurrence as tumor subpopulations. Going forward, quantitative and sensitive analyses of tumor mutations are needed to determine what level of KRAS mutation impacts patient response and acquired drug resistance.
AGE- AND SEX-RELATED ALTERATIONS IN RENAL GLOBAL DNA METHYLATION DURING THE LIFE CYCLE OF RATS

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Epigenetic modifications of DNA, such as methylation of cytosine, are critical to regulating gene and miRNA expression, cellular differentiation, and X-chromosome inactivation, and have been shown to be involved in human diseases including cancer. The role of epigenetic changes in renal development and diseases has been noted. However, comprehensive study is needed to understand the epigenetic effects on renal disease susceptibility and progression at different life stages and between the sexes. Recent advances in high throughput technologies such as high density methylation array and next generation sequencing make it possible to monitor the methylation status at the whole genome level. In this study, kidney tissues from Fischer 344 rats at 2, 5, 6, 8, 15, 21, 78, and 104wk of age for both sexes were examined for sex- and age-specific alterations in DNA methylation using Roche 385k rat promoter methylation microarrays, which cover 15,398 CpG island regions. The raw image data were analyzed by DEVA (v1.2.1) to compute the methylation changes in these samples. Biweight mean centering normalization was applied before downstream analysis.

Hierarchical clustering analysis indicated that the overall methylation patterns are similar between the age groups, but there are distinct methylation patterns between male and female rats. The results indicate that there are slightly more methylated sites at 2wk of age compared to later ages. A higher number of methylated sites were found on the X chromosome in females than males, suggesting involvement in repression of X-inactivated genes. These results provide a comprehensive global view of the DNA methylation status in the kidney over the entire rat life cycle. These age- and sex-related differences in DNA methylation may provide insights in susceptibility to kidney disease and its progression.
Oxybenzone is a compound that is commonly used in a variety of cosmetic products, especially sunscreens, for UV protection. Some in vitro studies have suggested that oxybenzone may have an estrogenic effect. The compound is detected in approximately 95% of tested human urine samples as well as in urine from premature infants. Recent studies reported that other UV-filters (benzophenone-2 and -4) have adverse effects on reproduction and development in fish and mammals due to their estrogen-like activity. To determine if fetal exposure to oxybenzone adversely affects testes development in rats, time-mated 12-13-week-old female Harlan Sprague-Dawley rats were dosed with 0, 1,000, 3,000, 10,000, 25,000, or 50,000 ppm oxybenzone (7-8 per group) added to chow from gestation day (GD) 6 until sacrifice at postnatal day (PND) 23. Reproductive organs were collected from PND 23 male pups. This study found body and testis weights decreased in a dose-dependent manner; however, there was not a difference in the testes:body weight ratio. Nevertheless, the number of spermatocytes per seminiferous tubule was significantly reduced accompanied by an increase in the number of apoptotic cells in males dosed with 3,000 ppm oxybenzone and higher. Serum testosterone levels were significantly decreased in the 3000, 25,000 and 50,000 ppm dosed groups. Some transcript levels in the steroidogenic pathway were decreased in testes at GD 20. Interestingly, these transcript levels were increased at PND 23. These results suggest that fetal exposure to oxybenzone may have adverse effects on steroidogenesis and spermatogenesis in male rats.
ROLE OF NEURAL STEM CELL ACTIVITY IN POSTWANEING DEVELOPMENT OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA IN RATS

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Abstract Here, we investigated the continued development of the SDN-POA in the postweaning period and whether stem cell activity might be involved in this later development. Methods. Brain blocks that included the SDN-POA were harvested from PND21 and PND110 Sprague-Dawley rats (6/sex/age). The SDN-POA outline was delineated using CB28 as a marker and verified by the DAPI-labeling method. Using adjacent brain slices, stem cell activity was visualized with an immunofluorescent triple labeling method: nestin or Ki67, CB28, and the DAPI nuclear labeling method. Results. Volumes of the female SDN-POA were 1.71±0.20x10⁻³ at PND 21 and 2.70±0.45x10⁻³ mm³ at PND 110, with no significant difference between the two ages. Male SDN-POA volumes were 4.91±0.48x10⁻³ and 7.03±0.45x10⁻³ mm³, respectively in the PND 21 and PND 110 groups. Both volumes were significantly larger than their same-age female group (p<0.01 for both) and the volume was larger in adults than in weanling males (p<0.05). In males, the number of Ki67-positive cells (indicating cell proliferation) in the SDN-POA area and the hypothalamus was 3.4 and 3.5 times higher, respectively (p<0.05 for both) in PND21 rats compared to PND110 rats. In females, the number was 1.7 and 3.3 times higher, respectively for the same regions (p>0.05 and p<0.05, respectively) in PND21 rats compared to PND110 rats. A subset of the Ki67-positive cells displayed cell dividing morphology in 3-dimensional views. Nestin-immunoreactivity delineated a potential macroscopic neural stem cell niche in the rostral end of the 3rd ventricle. In conclusion, Stem cells, likely derived from the stem cell niche adjacent to the 3rd ventricle, may account for some of this sexually dimorphic, post-weaning development of the SDN-POA. (Support: FDA NCTR P00710; NIH & UAMS funds)
ANESTHETIC-INDUCED NEUROTOXICITY IN THE INFANT NONHUMAN PRIMATE: 
MICROPET/CT IMAGING USING [18F]-FEPPA, A POTENTIAL BIOMARKER OF ASSOCIATED 
PATHOGENIC PROCESSES.

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Nitrous oxide (N2O) and isoflurane (ISO) are anesthetics commonly used in pediatrics. It is 
known that developmental exposures to a variety of anesthetics can cause abnormal 
neuronal cell death in animal models. Here, the effects of clinically-relevant exposures to N2O 
and ISO were examined in the rhesus monkey. Injury to the CNS is often accompanied by 
activation of microglia and the peripheral benzodiazepine receptor (PBR), a marker of 
activated microglia, has been reported to be a sensitive biomarker of neuroinflammation. 
Here, we monitored suspected inflammation induced by the N2O/ISO combination using a 
novel PET tracer for the PBR, [18F]-FEPPA. On postnatal day 5 or 6, animals were exposed to 
70% N2O, 29% oxygen and 1% ISO for 8 hours (n=4): control monkeys received room air only 
(n=4). One day, one week and three weeks later [18F]-FEPPA was injected iv and microPET/CT 
images were obtained over the next 2 hr. The radiotracer quickly distributed into the brains 
of all animals. One day after the N2O/ISO exposure the uptake of [18F]-FEPPA was significantly 
ingcreased in the Temporal Lobe (TL) of treated monkeys. A week later, uptake was 
significantly increased in the Frontal Cortex of treated animals but not in the TL. No 
significant differences between control and treated animals were seen at 3 weeks. This 
preliminary study demonstrated preferential uptake of a PBR ligand in the FC and TL of young 
animals exposed to N2O plus ISO, suggesting microglial activation lasting up to one week in 
those areas. [18-F]-FEPPA may serve as a translational biomarker of CNS inflammation. 
(supported by NCTR E# 7285)
EVALUATION OF WILD YAM (Dioscorea Villosa) ROOT EXTRACT AS A POTENTIAL EPIGENETIC AGENT IN BREAST CANCER CELLS

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Aberrant epigenetic alterations in the genome, is believed to be a potential cause of some forms of cancer. Due to their reversibility, epigenetic modifications are considered potentially useful in drug development approaches (epi-drugs). The current available synthetic epi-drugs are non-specific and induce adverse effects. Natural products might offer advantages and find utility for cancer treatment. The present study was designed to evaluate the efficacy of wild yam root extract as a potential demethylating agent using two breast cancer cell lines, MCF-7 (Estrogen receptor positive, ER+) and MDA-MB-231 (ER negative, ER-), and a gene, GATA-3, a potential marker of breast cancer development. Moreover, GATA-3 expression is methylation-specific, being higher in ER+ cells with promoter hypomethylation and insignificant in ER- with promoter hypermethylation. In this study, cells, approximately at 70 % confluency, were treated with wild yam root extract (0-50 μg/ml) for 72h and then used for viability, mRNA, and methylation analyses. It was observed that wild yam significantly reduced viability of both cell lines and enhanced the mRNA contents of DNMTs (DNMT1, 3A, and 3B) and GATA-3 in a dose-dependent manner. Global DNA methylation, analyzed as 5'-methyl-2'-deoxycytidine (mC) and 5-hydroxymethylcytosine (hmC), showed that mC was increased only in MCF-7 cells, whereas hmC level was reduced in both cell lines. Since hmC is generated from mC by ten-eleven-translocation (TET) enzymes, the present data suggest that enhanced expression of GATA-3 and DNMT enzymes followed by a reduction in hmC in MCF-7 and MDA-MB-231 cells are the result of interruption of TET enzyme functions in the epigenome by wild yam root extract. This plant with a long history of traditional use should be further explored with regard to its potential as an epigenetic agent in breast cancer therapy.
Maternal smoking substantially increases the risk of learning disabilities, behavioral problems, and attention deficit/hyperactivity disorder in offspring. Nicotine is the main pharmacologically active component of tobacco smoke. Prenatal exposure to nicotine is found to be deleterious to the developing brain and capable of causing fetal brain damage. To evaluate nicotine’s effects on the developing nervous system and explore associated mechanisms, rat embryonic neural stem cells were used.

Brain cortices were collected from fetal rats (gestational day 14, GD14) for neural stem cell isolation and subsequent culture in commercial rat growth medium in a humidified atmosphere. On the 8th day in vitro (DIV), confluent neural stem cells were exposed to nicotine at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0 and 10 μM for 24 hours. Neural stem cells were identified using a monoclonal anti-nestin antibody. Mitochondrial function (MTT assays), was monitored to determine the effects of nicotine on neural stem cell proliferation. Immunohistochemical staining of active caspase 3, TUNEL assays and annexin V - propidium iodide (PI) staining were employed to assess neural stem cell apoptosis. To determine if mitochondrial dysfunction contributed to the neural stem cell death, JC-1 staining was conducted.

Nicotine exposure dose-dependently affected mitochondrial function, indicated by the MTT assay. Nicotine also increased active caspase 3 expression. Both annexin V – PI staining and TUNEL assays indicated nicotine-induced apoptosis. JC-1 staining suggested that nicotine affected mitochondrial membrane potentials, which could be a route leading to apoptosis. These observations suggest that nicotine decreased neural stem cell viability, which can affect the development of the central nervous system and contribute to behavioral problems in offspring. Supported by NCTR/FDA (Experiment E-7417).
Bisphenol A (BPA), a synthetic chemical present in a wide variety of consumer products, has received considerable attention throughout the last decade because of its widespread exposure to people. A physiologically based pharmacokinetic (PBPK) model for BPA was developed in adult and neonatal rats to quantitatively evaluate age-dependent pharmacokinetic behavior of BPA and its phase II metabolites. The BPA PBPK model was calibrated using published BPA studies on in vitro hepatic and intestinal metabolism of BPA, in situ biliary excretion of BPA metabolites formed in the liver, and fecal and urinary excretion and serum time course data collected after oral and intravenous administration of BPA. Metabolism of BPA in the small intestine and liver was responsible for the low oral bioavailability of BPA and enterohepatic recirculation was predicted to prolong the systemic levels of both BPA and its metabolites. The model-predicted area under the serum concentration time curve and peak serum concentration for BPA after oral administration were 11-fold and 122-fold greater, respectively, in PND3 pups than in adults. The BPA PBPK model, calibrated for aqueous administration of 100 μg/kg of BPA in young and adult rats, was evaluated by simulating data in young and adult rats administered 1 or 10 mg/kg of BPA dissolved in corn oil (Domoradzki et al., 2004). Model predictions were inconsistent with observations. Adjustments in model parameters suggest that a recalibration of the model would be required to fit these data with consideration of dosing vehicle and dose-dependent behavior involving the gastrointestinal tract.
MAGNETIC RESONANCE IMAGING AND SPECTROSCOPIC MARKERS OF KAINIC ACID-INDUCED EXCITOTOXICITY IN THE RAT BRAIN

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In this study we describe changes in brain after the acute administration of kainic acid (KA) using magnetic resonance imaging (MRI) and spectroscopy (MRS) in comparison to histopathology. Adult male Sprague-Dawley rats (N = 24) were anesthetized with isoflurane and positioned inside a 7 tesla MRI scanner. T₂ relaxation mapping of the whole brain and ¹H-MRS in the left hippocampus were performed. KA (10 mg/kg, ip) was then administered to all animals after baseline scans and imaging was continued for another 2 hours. One group of animals (N = 6) was euthanized at this time. The MRI procedure was repeated one day (N = 6) and 2 days later (N = 12) for different groups of animals, which were euthanized immediately after the scan. All rats were perfusion-fixed for further histological assessment. KA led to an increase in T₂ in the hippocampus as early as 1 hour after administration. These changes were more pronounced at 2 hours and drastically so at 1 and 2 days after the treatment at which point the findings spread to wider areas of the brain, including the amygdale, thalamus, and cortex. MRS revealed immediate increases in glutamate and glutamine concentrations right after KA administration followed by decreases at 1 and 2 days, at which point the N-acetyl-aspartate signal was also decreased. Lactate was not detectable in the normal brain but appeared at 15 min and increased to a maximum at 2 hours after treatment. At 1 and 2 days lactate was still detectable. The T₂ water relaxation correlated with histological changes in the brain. These data provide the basis for the development of imaging biomarkers of neurotoxicity. (Supported by NCTR and CDER, FDA, #E0741801).
Saffron extracts have induced apoptosis, cell cycle arrest, inhibited cellular proliferation, and tumor progression in various cancer cell lines. We are interested in studying the potential chemopreventative effects of saffron especially as it relates to prostate cancer. Recognized active constituents of saffron are crocetin and safranal. Cytotoxicity of safranal was investigated using the androgen responsive 22Rv1 prostate cancer cell line. The cytotoxicity IC50 of safranal at 24 hr was 141 μM using the tetrazolium dye assay (XTT). The assay was incompatible with crocetin. Using the Caspase-Glo® 3/7 assay system, it appears that apoptotic mechanisms were involved in safranal’s cytotoxicity because after 6 hr of exposure, the EC50 of apoptosis was similar to the cytotoxicity IC50. Safranal’s antioxidant activity as measured by a 2’,7’-dichlorodihydrofluorescein diacetate assay indicated decreased reactive oxygen species formation. Ongoing studies are investigating the potential of saffron to also inhibit prostate cell invasion and migration in vitro. (Supported by Saudi Arabian Ministry of Higher Education and Salman bin Abdulaziz University)
L-CARNITINE AMELIORATES PROPOFOL-INDUCED TOXICITY IN RAT EMBRYONIC NEURAL STEM CELLS


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Propofol is a widely used anesthetic agent for adults and children. A growing body of data suggests that exposure to anesthetics during certain periods of perinatal development has long-term deleterious effects. At the cellular level there is evidence in the developing brain that anesthetic agents induce cell death, cause synaptic remodeling, and alter brain cell morphology. L-carnitine, an anti-oxidant dietary supplement, has been reported to prevent neuronal damage from a variety of causes in vitro and in vivo. To evaluate the ability of L-carnitine to protect against propofol-induced neuronal toxicity, a rat embryonic neural stem cell model was used.

Brain cortices were collected from fetal rats [gestational day (GD) 14] for neural stem cell isolation and subsequent culture in commercial rat growth medium in a humidified atmosphere. On the 8th day in vitro (DIV), confluent neural stem cells were exposed to propofol at concentrations of 10, 50, 100, 300 and 600 μM or propofol plus acetyl-l-carnitine (10 μM) for 24 hours. Neural stem cells were identified using monoclonal anti-nestin antibody. Markers of cellular proliferation (EdU), mitochondrial health (MTT), cell death/damage (LDH) and oxidative damage (8-oxo-dG) were monitored to determine: 1) the effects of propofol on neural stem cell proliferation; 2) the nature of propofol-induced neurotoxicity; 3) the degree of protection afforded by acetyl-l-carnitine; and 4) to provide information regarding potential underlying mechanisms.

The EdU data demonstrated that after exposure to propofol for 24 hrs at a clinically-relevant concentration (50 μM), the number of dividing cells was significantly decreased. Propofol exposure also resulted in a substantial dose-dependent reduction in mitochondrial health as evidenced by significant decreases in the metabolism of MTT. No significant effect on LDH release was observed at propofol concentrations of 10, 50 or 100 μM. 50 μM propofol significantly increased oxidative DNA damage as evidenced by increases in 8-oxo-dG formation and this effect was blocked by acetyl-l-carnitine. No significant effect on 8-oxo-dG formation was observed when acetyl-l-carnitine was administered alone.

These data suggest that clinically-relevant concentrations of propofol induce dose-dependent adverse effects on rat embryonic neural stem cells: 24 h exposures slow or stop cell division/proliferation and cause cellular damage. The presence of elevated levels of 8-oxo dG and its analogs in the culture medium suggest the occurrence of oxidative damage due to increased generation of reactive oxygen species. Co-administration of acetyl-l-carnitine effectively blocks at least some of the toxicity of propofol, presumably by scavenging ROS and/or reducing ROS production.

Supported by NCTR/FDA (Experiment E-7417)
Chemoprevention has been a pivotal and effective strategy during the skin cancer treatment. Using human skin normal and tumor samples, we demonstrated that both the expression and activity levels of pyruvate kinase M2 (PKM2) were higher in skin tumor tissues than normal tissues, suggesting that PKM2, one of important metabolic enzyme, might serve as a target for skin cancer prevention and/or therapy. Shikonin, a small-molecule active chemical, has been studied as an anti-cancer drug candidate in human cancer models. However, the mechanism of action and the chemopreventive potential of shikonin are unclear. Herein, we used the skin epidermal JB6 P+ cells and demonstrated that shikonin suppressed the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA) induced neoplastic cell transformation using soft agar assay. We demonstrated that shikonin inhibited TPA-induced PKM2 activation during the early stage of carcinogenesis as well. Moreover, Mitochondrial functions were inhibited by TPA treatment, as indicated by reduced mitochondrial membrane potential and mitochondrial respiration, which were restored by shikonin. We also examined the levels of lactate as a glycolysis marker, and shikonin suppressed its increase caused by tumor promoter treatment. Modulation of cell metabolism by shikonin was associated with G2-M phase accumulation, and Fra-1 (a major subunit of activator protein 1 in skin tumorigenesis) downregulation. Interestingly, we found that AMP -activated protein kinase (AMPK), an energy sensor, which is inactivated by TPA, shikonin could reverse AMPK activity. These results suggest that shikonin bears chemopreventive potential for human skin cancers in which PKM2 is upregulated, which might be mediated by inhibiting oncogenic activation, PKM2 activation, mitochondrial dysfunction, and activated AMPK.
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NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS INDUCE PREMATURE SENESCENCE IN AORTIC ENDOTHELIAL CELLS.

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HIV patients undergoing antiretroviral therapy exhibit an increased incidence of cardiovascular events and their associated diseases. Though HIV therapy generally involves a combination drug approach, nucleoside reverse transcriptase inhibitors (NRTI) are considered a backbone of this therapy. Prior studies in rodents suggested that NRTI promote HIV-associated endothelial dysfunction, an initiating factor in atherosclerosis. Further, this cellular dysfunction was associated with mitochondrial injury. In cultured endothelial cells, NRTI treatment increased mitochondrial ROS production, while decreasing ATP production and the activities of mitochondrial electron transport chain (ETC) complexes I-IV. While these effects were observed for acute treatment, in other studies, we noted that when the mitochondria were exposed to low doses of NRTI, damaged mitochondria were removed through a process known as mitophagy. Thus, our hypothesis was that with chronic treatment, this repair mechanism may be overwhelmed, so as to promote premature endothelial senescence. In these studies, human aortic endothelial cells (HAEC) were exposed to chronic NRTI treatment. ROS production, cell proliferation rate and levels of senescence were determined. Our findings were that NRTI treatment increased ROS production, at least initially. Also, the rate of cell proliferation decreased from passage 1 to passage 8, and the level of NRTI-induced senescence increased. In addition, co-treatment with the mitochondrial antioxidant coenzyme (Q10) resulted in a delayed onset of senescence. In conclusion, long term NRTI treatment resulted in ROS production, reduced cell proliferation rate and premature endothelial senescence. Moreover, our findings may suggest approaches for reducing the cardiovascular side effects of NRTI therapy.
SHORT-TERM AND CHRONIC EXPOSURES OF CIGARETTE SMOKE CONDENSATE (CSC) INDUCE DIFFERENTIAL EXPRESSION AND PROMOTER METHYLATION PROFILES OF CRITICAL GENES INVOLVED IN LUNG CANCER: In Vitro ANALYSIS OF POTENTIAL HARM OF TOBACCO SMOKE IN LUNG.

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Etiologically, tobacco smoking represents the single most important risk factor for lung cancer development. Continued efforts are, however, necessary to better define the mechanisms contributing to tobacco-induced pulmonary carcinogenesis, and to identify novel molecular targets for the treatment and prevention of this tobacco-related disease, as well as molecular markers for the assessment of risk. This study examined the effects of cigarette smoke condensate (CSC) in vitro on expression and promoter methylation profile of critical genes involved in various stages of lung cancer development, such as death-associated protein kinase (DAPK), E-cadherin (ECAD), O6-methyl-guanine-DNA methyltransferase (MGMT), and RASSF1A. NL-20 lung cells were treated with 0.1, 1, 10 or 100 μg/ml of CSC for 24, 48 and 72 hrs for short-term exposures. DAPK expression was not significantly affected, nor was its methylation status. However, ECAD showed a significant decrease in expression after 72 hrs with both 10 and 100 μg/ml CSC. This decrease in expression correlated to hypermethylation of the ECAD promoter at both doses using MSP analysis. MGMT expression increased only with the dose of 100 μg/ml CSC at all time points. This correlated to an unmethylated profile of the promoter. RASSF1A promoter profile was methylated in the control samples. However, when treated with 10 or 100 μg/ml CSC, an unmethylated promoter profile was noted. This correlated to increased expression of RASSF1A at both the 10 and 100 μg/ml doses. No changes were noted at 0.1 or 1 μg/ml of CSC in NL-20 lung cells. For chronic studies, the cells were exposed to 1 or 10 μg/ml CSC for 7, 14, or 28 days, with media changes every three days adding new CSC. After 28 days, cells showed morphological changes associated with transformation. Foci were noted. These cells were further analyzed for invasion capacities and global methylation status. This study provides critical data showing epigenetic regulation of critical genes involved in DNA repair, invasion and tumor suppressor with CSC treatment of lung cells.
INFLUENCE OF AGE AND SEX ON MITOCHONDRIA-RELATED GENE EXPRESSION IN RAT HEART.

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The role of mitochondria was investigated for age- and sex-related differences in rat heart. Gene expression in the heart of male and female Fischer 344 rats at 8 (young), 21 (adult) and 78 (old) weeks of age was measured using Agilent whole genome rat arrays. Out of 18,435 unique genes on the Agilent rat microarray, 869 unique genes were determined to be mitochondria-related. Age effect was evaluated by using ANOVA coupled with pair-wise t-test (p<0.05) between each age group, and sex difference was evaluated at each age using t-test (p<0.05). The expression levels of genes involved in oxidative phosphorylation (Ox Phos), membrane transporters, and fatty acid (FA) metabolism were the highest at 21 wks compared to 8 or 78 wks in both males and females. A significant age related effect was observed in 21% and 7% of Ox Phos genes and 15% and 7% of membrane transporter genes in males and females, respectively, at all age groups. Significant sex-based differences were observed in the expression levels of genes involved in Ox Phos at 78 wks (19%); higher expression levels were noted in female rats compared to males. Membrane transporters showed significant sex differences at 8 wks (21%), 21 wks (40%) and 78 wks (13%). Genes involved in FA metabolism also showed sex based differences at 8 (34%), 21 wks (29%) and 78wks (10%); most of the genes had higher expression in females than males. The inherent differences in the expression levels of mitochondria-related genes at various ages and between sexes may help us understand the mitochondrial association with cardiac diseases in diverse population.
EFFICACY AND SAFETY OF CHRONIC ANTI-(+) METHAMPHETAMINE MAB7F9 TREATMENT OF (+)- METHAMPHETAMINE (METH) INDUCED LOCOMOTOR ACTIVITY IN RATS

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These studies examined how chronic anti-METH monoclonal antibody therapy affects METH-induced locomotor activity. We hypothesized that chronic anti-(+)-METH mAb7F9 treatment would reduce METH-induced behaviors in a safe and sustained manner. Rats were conditioned to 0.56 mg/kg iv METH. After each dose, distance traveled was measured for 4 hrs. For the experiments, rats were treated with saline or an iv 282 mg/kg mAb7F9 loading dose with two weekly 141 mg/kg maintenance doses, which provided one-half equimolar mAb7F9 binding sites to the METH dose. METH doses were administered 4 hrs and 3 days after each of the three mAb7F9/control treatments, and 7 days after the last treatment. Serum samples were collected 5 hrs after each METH dose for LC–MS/MS analysis. At 4 hrs after each mAb7F9 administration and 3 days after the first administration, METH-induced locomotor activity was significantly reduced. While the locomotor data suggested attenuation in mAb7F9 effects over time, the serum METH concentrations showed substantive differences between vehicle- and mAb7F9-treated rats after each METH dose. We examined mAb7F9 safety and efficacy at 10 and 14 days after the final mAb7F9 dose in the same rats using binding capacity exhausting, 3-fold higher METH doses (1.68 mg/kg). mAb7F9 treatment led to a >90-min earlier termination of the METH-induced locomotor effects relative to controls. Even in the presence of repeated METH challenge doses, chronic mAb7F9 treatment appears to be efficacious and safe. (Support: NIDA grants DA 11560 and U01 DA23900)
KIDNEY miRNAs SHOW AGE AND SEX DIFFERENCES IN EXPRESSION DURING THE RAT LIFE CYCLE

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Increasing evidence for epigenetic mechanisms of gene regulation has fueled interest in miRNAs in toxicogenomics for biomarker discovery. While still immature compared to other genomic resources, growing knowledge of individual miRNAs and their putative gene targets allows for large scale inquiry into genome-wide analysis of miRNA expression. F344 rats were used to evaluate kidney miRNAs with putative roles in drug metabolism and kidney disease. miRNA expression was characterized at 2, 5, 6, 8, 15, 21, 78, and 104 weeks of age in both sexes (n=5) using Agilent 8x15k rat miRNA microarrays. 224 miRNAs were found to be expressed in the kidney in at least one age and sex. Filtering criteria of 1.5 fold change and p<0.05 (2-way ANOVA) revealed 105 miRNAs (47%) exhibiting differential expression. Principal component analysis (PCA) showed PC1 accounted for 21% of the variability in a pattern consistent with age-specific effects. 12 miRNAs showed increased expression at 78 and 104 weeks, consistent with an aging-related effect (e.g. miR-142-3p, miR-223). Although no large scale, sex-related patterns were evident from the PCA, some miRNAs showed sex-specific patterns (e.g. miR-204, miR-499, miR-183). miR-499 has been implicated in regulation of mitochondrial dynamics through direct targeting of calcineurin. Collectively, these results comprise one of the first large-scale characterizations of global miRNAs in the kidney over the rat life cycle. Age- and sex-related differences were observed that may impact susceptibility to adverse effects in the kidney. (Support: US FDA)
IN VIVO EFFECTS OF ABUSED “BATH SALT” CONSTITUENT 3,4-METHYLENEDIOXYPYROVALERONE (MDPV) IN MICE: DRUG DISCRIMINATION, THERMOREGULATION, AND LOCOMOTOR ACTIVITY

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In recent years, synthetic analogues of naturally-occurring cathinone have emerged as psychostimulant-like drugs of abuse in commercial “bath salt” preparations. 3,4-methylenedioxypyrovalerone (MDPV) is a common constituent of these illicit products, and its structural similarities to the more well-known drugs of abuse 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine (METH) suggest that it may have similar in vivo effects to these substances. In these studies, adult male NIH Swiss mice were trained to discriminate 0.3 mg/kg MDPV from saline, and the interoceptive effects of a range of substitution doses of MDPV, MDMA and METH were then assessed. In separate groups of mice, surgically-implanted radiotelemetry probes simultaneously monitored thermoregulatory and locomotor responses to various doses of MDPV and MDMA, as a function of ambient temperature. We found that mice reliably discriminated the MDPV training dose from saline, and that cumulative doses of MDPV, MDMA, and METH all fully substituted for the MDPV training stimulus. All three drugs had similar ED₅₀ values in this procedure. Stimulation of motor activity was observed following administration of a wide range of MDPV doses (1 to 30 mg/kg), and the warm ambient temperature potentiated motor activity, and elicited profound stereotypy and self-injurious behavior at 30 mg/kg. In contrast, similar MDPV-induced hyperthermic effects were observed in both the cool and warm ambient environments. This pattern of effects is in sharp contrast to MDMA, where ambient temperature interacts with thermoregulation, but not locomotor activity. These studies suggest that while the interoceptive effects of MDPV are similar to those of MDMA and METH, direct effects on thermoregulatory processes and locomotor activity are likely mediated by different mechanisms than those of MDMA. (Support: UAMS Center for Translational Neuroscience (RR020146) and the UAMS Translational Research Institute (RR029884))
MITOCHONDRIAL BIOGENESIS AND MITOPHAGY FOLLOWING MANGANESE SUPEROXIDE DISMUTASE KNOCKDOWN


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Oxidative stress caused by reactive oxygen species (ROS) can lead to chronic conditions such as neurodegenerative and cardiovascular diseases, as well as aging and cancer. It can also play a major role in acute conditions such as multi-organ dysfunction in sepsis. One source of endogenous ROS is the mitochondrial electron transport chain (ETC). Manganese Superoxide Dismutase (MnSOD) is the major mitochondrial antioxidant, and it catalyzes the transformation of superoxide into hydrogen peroxide which can then be further detoxified by other cellular enzymes. MnSOD is critical in maintaining the normal function of mitochondria. Using a siRNA mediated MnSOD knockdown of mouse collecting duct (mIMCD3) cells as the in vitro model, and a kidney-specific MnSOD knockout (KO) mouse as the in vivo model, our preliminary data show evidence of increased mitochondrial biogenesis and mitophagy. We hypothesize that loss of MnSOD activity leads to compensatory mitochondrial biogenesis, as well as time-dependent mitophagy. We also used primary cultures of mouse cortical and medullary tubular epithelial cells exposed to septic serum as an in vitro model to study the function of MnSOD in the progression of sepsis. Preliminary data show increased autophagy and MnSOD expression following exposure to septic serum. The goal of this project is to better understand the sequence of molecular events following MnSOD knockdown. This knowledge will identify novel target(s) and pathway(s) for therapeutic approaches. (Support: 1RO1DK0789361)
Traditionally, chlorpyrifos (CPS) mediates its toxicity through inhibition of cholinesterase (ChE). However, in recent years, the toxicological effects of developmental CPS exposure have been attributed to an unknown non-cholinergic mechanism of action. We hypothesize that the endocannabinoid system may be an important target because of its vital role in nervous system development. We have previously reported that repeated exposure to CPS results in greater inhibition of the fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), than inhibition of either ChE or monoacylglycerol lipase (MAGL), the enzyme that metabolizes the endocannabinoid 2-arachidonylglycerol (2-AG). This exposure resulted in the accumulation of AEA in the forebrain of juvenile rats, but even at the lowest dosage level used (1.0 mg/kg) ChE inhibition was still present. Thus, it was not clear if FAAH activity will be inhibited as dosage levels that do not inhibit ChE. To determine this, 10 day old rat pups were exposed daily for 7 days to either corn oil or 0.5 mg/kg CPS by oral gavage. At 12 hrs post-exposure, the activities of ChE, MAGL, and FAAH were determined in the forebrain, as well as the levels of the endocannabinoids AEA and 2-AG. There was no significant inhibition of the activities of ChE or MAGL and no significant change in the amount of 2-AG. In contrast, FAAH activity was significantly inhibited resulting in a marked accumulation of AEA in the forebrain. Although it has not been determined whether this alteration of endocannabinoid signaling can impact brain maturation, it does suggest a potential candidate for the non-cholinergic mechanism of action of CPS.
ASIAN GINSENG (PANAX GINSENG) POTENTIATES ETHANOL-INDUCED CARDIOVASCULAR DYSFUNCTION IN MEDAKA EMBRYOGENESIS (ORYZIAS LATIPES)

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Alcohol is a teratogen, induces fetal alcohol spectrum disorder (FASD) which has serious central nervous system (CNS), cardiovascular, and craniofacial defects affecting the entire lifetime of an individual. Prevention of FASD, other than women abstaining from drinking alcohol during pregnancy, is not known. The synthetic drugs recommended for the treatment of alcoholism cannot be used by women during pregnancy which led us to investigate on natural products. Due to ethical constraints, FASD studies in humans are very limited and several animal models are used to understand the molecular mechanisms. We have observed that developmental ethanol exposure of medaka (Oryzias latipes) embryos generates features which are analogous to human FASD phenotypes. We hypothesize that ethanol metabolism generates oxidative stress which can disrupt embryonic development of medaka. In the present experiment, we have used root extracts of Asian ginseng (Panax ginseng) as a preventive agent of FASD. Fertilized medaka eggs within 4 h post fertilization (hpf) were exposed to methanolic extracts (50-100 ug/ml) of ginseng root (PG) or ethanol (300 mM) either alone or in combination. After 48 h of treatment the viable embryos were transferred to clean hatching solution and on 6 dpf the embryos were examined for vessel circulation followed by mRNA analyses of enzymes related to ethanol metabolism and oxidative stress. It was observed that ethanol (300 mM) alone was able to disrupt vessel circulation and cotreatment of PG (50-100 ug/ml) with ethanol enhanced the effect; PG (100 μg/ml) alone has no effect. mRNA analysis of alcohol metabolizing enzymes or oxidative stress-related enzymes did not show any significant alterations in any of these treatment conditions. It is therefore concluded that potentiation of ethanol-induced cardiovascular deformities in medaka by PG may be mediated through a different mechanism rather than oxidative stress.
Acute DFP intoxication leads to long-term neurobehavioral changes (depressive-like behavior) in rats. We hypothesized that early alterations in serotonin signaling contribute to later neurochemical and behavioral changes following DFP. Male rats were treated with either vehicle or DFP (2.25 mg/kg, sc) and then given vehicle or combined URB597 (3 mg/kg)/URB602 (10 mg/kg, ip), i.e., inhibitors of endocannabinoid (eCB) degrading enzymes. Rats were sacrificed at 4h, 1d, 7d or 30d and cortex was dissected for HPLC-ED analysis. 5-HT was reduced 17% 4h after DFP, but slightly elevated with DFP + URB597/URB602 (URBs). 5-HIAA increased dramatically (about 65%) 4h after DFP and was further increased (about 110%) 4h after DFP + URPs. By 1d, 5-HT returned to control levels with DFP only whereas 5-HT was still elevated (18%) in rats given DFP + URBs. At 7d, rats treated with DFP only and DFP + URBs showed about 12% reduction in 5-HT. A similar reduction in 5-HIAA was also noted with DFP only, but 5-HIAA was still elevated (17%) 7d after DFP + URBs. By 30d, all treatment groups showed similar but slightly reduced 5-HT and 5-HIAA levels. As we previously reported that under similar dosing conditions, URPs blocked DFP-induced behavioral changes, the results suggest serotonin signaling contributes to the neurobehavioral sequelae and that eCB signaling can modulate neurochemical and behavioral changes elicited by DFP. (Supported by NIEHS R01 ES009119).
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