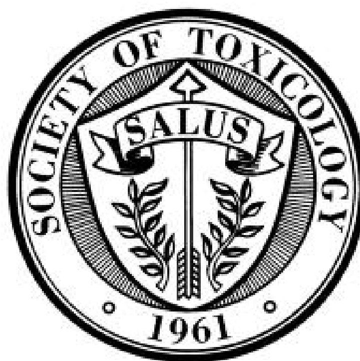
29th Annual Meeting of the Mountain West Society of Toxicology

"Oxidative Stress, Inflammation and Human Disease"

September 8-9, 2011
Breckenridge, CO

Hosted by the Department of Pharmaceutical Sciences and the Molecular Toxicology Program, School of Pharmacy at the University of Colorado Denver

Mountain West Society of Toxicology



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29th ANNUAL MOUNTAIN WEST SOCIETY OF TOXICOLOGY

Meeting Agenda

Thursday, September 8th, 2011

7:30 – 8:30 am Pick-up Registration Materials
Location: Imperial Foyer

7:30 – 8:30 am Continental Breakfast
Location: Imperial Foyer

8:00 – 8:30 am Housekeeping and Introduction
Location: Imperial Ballroom
Cynthia Ju, PhD and Donna Zhang, PhD (MWSOT Vice-President and President)

8:30 – 9:15 am KEYNOTE PRESENTATION
"INFLAMMATION, LIVER REGENERATION, AND LIVER CANCER: A KEY ROLE OF INTERLEUKIN-22"

Bin Gao, NIAAA, National Institutes of Health

9:15 – 10:45 am PLENARY TOPIC I: ENVIRONMENTAL INSULT-INDUCED OXIDATIVE STRESS, INFLAMMATION, AND DISEASE --- Chair: David Ross

9:15 – 9:45 am
"ACTIVATION OF TRPA1, V1, AND M8 BY COMBUSTION-DERIVED PARTICULATE MATERIALS: RELATIONSHIP TO LUNG INFLAMMATION AND INJURY", **Christopher Reilly**, University of Utah

9:45 – 10:15 am
"SYNERGISTIC IMMUNOSUPPRESSION BY ARSENIC AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHS): THE POWER OF TWO", **Scott Burchiel,** University of New Mexico

10:15 – 10:45 am
"ARSENIC-INDUCED AUTOPHAGY: LIFEBOAT OR TORPEDO", **Walter Klimecki,** University of Arizona

10:45 – 11:15 am Coffee Break
Location: Imperial Foyer

11:15 – 12:45 pm STUDENT AND POST-DOCTORAL PRESENTATIONS -- Chair: Serrine Lau

11:15 – 11:30 am
"ARSENIC ALTERS BARRIER PROPERTIES IN AIRWAY EPITHELIAL CELLS", **Cara Sherwood,** University of Arizona

11:30 – 11:45 am
"PROFILING PROTEIN CARBONYLATION IN A MURINE MODEL OF ALCOHOLIC LIVER DISEASE", **James Galligan,** University of Colorado Denver

11:45 – 12:00 pm
"ARSENITE INTERACTS SELECTIVELY WITH ZINC FINGER PROTEINS CONTAINING C3H1 OR C4 MOTIFS", **Xixi Zhou** (PhD), University of New Mexico

12:00 – 12:15 pm
"COMPARATIVE BIORESPONSES OF QUANTUM DOT SIZE, CHARGE AND FUNCTIONALIZATION IN PRIMARY HUMAN LUNG EPITHELIAL CELLS", **Amber Nagy** (PhD)**,** Los Alamos National Laboratory

12:15 – 12:30 pm
"ANALYSIS OF ENDOPLASMIC RETICULUM (ER) STRESS-RELATED LIPOGENESIS GENES IN PROGRESSIVE HUMAN NONALCOHOLIC FATTY LIVER DISEASE", **April Lake**, University of Arizona

12:30 – 12:45 pm
"THE ROLE OF ALDH1B1 IN ALCOHOL METABOLISM, COLON CARCINOGENESIS AND DIABETES: CHARACTERIZATION OF *Aldh1b1* NULL MICE", **Surrendra Singh**, University of Colorado Denver

12:45 – 2:30 pm MWSOT Officers Luncheon (by invitation)
Location: Coppertop III

12:45 – 2:30 pm Lunch and Free Time

2:30 – 5:00 pm PLENARY TOPIC II: CONDUCTING TOXICOLOGICAL STUDIES IN DRUG INDUSTRY --Chair: Cynthia Ju

2:30 – 3:00 pm
"PHARMACEUTICAL TOXICOLOGY - INTEGRATION OF REGULATORY REQUIREMENTS, MECHANISTIC INVESTIGATIONS AND RISK ASSESSMENT", **Timothy Reilly**, Bristol-Myers Squibb

3:00 – 3:30 pm
“IMMUNOTOXICITY TESTING STRATEGIES IN PHARMACEUTICAL DRUG DEVELOPMENT”, **Mark Collinge**, Pfizer, Inc.

3:30 – 4:00 pm Coffee Break
Location: Imperial Foyer

4:00 – 4:30 pm
“DISCOVERY & INVESTIGATIVE TOXICOLOGY: LEAD OPTIMIZATION & ISSUE INVESTIGATION TO ENABLE CANDIDATE IDENTIFICATION & RATIONALE DECISION-MAKING”, **Donna Dambach**, Genentech, Inc.

4:30 – 5:00 pm
“DRUG METABOLISM AND IDENTIFYING COMPOUND LIABILITY AND ITS IMPACT IN DRUG DEVELOPMENT: A CASE STUDY**”, Michael Wempe**, University of Colorado Denver

5:00 – 5:30 pm Poster Set-Up
Location: Peaks 14-16

5:30 – 7:30 Poster Session

7:30 – 9:00 Banquet Buffet Dinner
Location: Peak 17

Friday, September 9th, 2011

7:00 – 8:30 Continental Breakfast
Location: Imperial Foyer

8:30 – 10:00 am PLENARY TOPIC III:OXIDATIVE STRESS, INFLAMMATION AND LIVER DISEASE --Chair: Vasilis Vasiliou

8:30 – 9:00 am
“MATERNAL HIGH FAT DIET PROGRAMS FETAL HEPATIC LIPOTOXICITY AND EARLY METABOLIC DISEASE IN THE NON-HUMAN PRIMATE”, **Jed Friedman,** University of Colorado Denver

9:00 – 9:30 am
“TREATMENT OF CHRONIC HEPATITIS C WITH FIRST GENERATION NS3/4A PROTEASE INHIBITORS”, **Gregory Everson,** University of Colorado Denver

9:30 – 10:00 am
"HEPATOCYTE-SPECIFIC PROTECTION BY A2B ADENOSINE RECEPTORS DURING ISCHEMIA- REPERFUSION INJURY OF THE LIVER", **Michael Zimmerman,** University of Colorado Denver

10:00 – 10:30 am Coffee Break
Location: Imperial Foyer

10:30 – 12:00 pm STUDENT AND POST-DOCTORAL PRESENTATIONS -- **Chair: Matt Reed**

10:30 – 10:45 am
"TUNING OF STRESS RESPONSE BY GLUTATHIONE IN STEATOHEPATITIS", **Ying Chen** (PhD)**,** University of Colorado Denver

10:45 – 11:00 am
"ACTIVATION OF THE IRRITANT RECEPTOR TRPA1 BY WOOD SMOKE PARTICULATE MATERIAL AND ASSOCIATED CHEMICAL COMPONENTS", **Darien Shapiro,** University of Utah

11:00 – 11:15 am
"4-HNE SIGNIFICATLY ALTERS L-FABP STRUCTURAL AND FUNCTIONAL DYNAMICS", **Becky Smathers,** University of Colorado Denver

11:15 – 11:30 am
"THERAPEUTIC POTENTIAL OF NRF2 ACTIVATORS IN STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY", **Samantha Whitman** (PhD), University of Arizona

11:30 – 11:45 am
"ARSENITE INDUCES ENDOPLASMIC RETICULUM STRESS AND THE UNFOLDED PROTEIN RESPONSE IN LYMPHOBLASTOID CELLS", **Fei Zhao,** University of Arizona

11:45 – 12:00 pm
"GENE DELETION OF NOS2 PREVENTS MANGANESE-INDUCED NEUROLOGICAL INFLAMMATION IN DEVELOPING MICE", **Karin Streifel,** Colorado State University

12:00 – 12:15 pm Break and Awards Committee Meeting

12:15 – 12:30 pm Presentation Awards and Closing Remarks
Cynthia Ju, PhD and Donna Zhang, PhD (MWSOT Vice-President and President)

Speaker Abstracts

SYNERGISTIC IMMUNOSUPPRESSION BY ARSENIC AND POLYCYCLIC AROMATIC HYDROCARBONS (PAHS): THE POWER OF TWO

Scott W. Burchiel
The University of New Mexico College of Pharmacy, Toxicology Program, Albuquerque, NM 87131

Many polycyclic aromatic hydrocarbons (PAHs) are important environmental carcinogens that also produce immunosuppression in animals and humans. My laboratory has shown that benzo(a)pyrene (BaP) and 7,12-dimethylbenzanthracene (DMBA) produce immunotoxicity via genotoxic events requiring metabolic activation by CYP1A1 and/or CYP1B1, followed by metabolism by microsomal epoxide hydrolase (EPHX1), and a second round of metabolism by CYP1A1/CYP1B1resulting in the production of diol-epoxides. Our work has shown that DMBA is not immunosuppressive in CYP1B1 null and EPHX1 null mice. In addition, p53 null mice are resistant to immunotoxicity produced by DMBA, and we have demonstrated that ATR and ATM play key roles in genotoxicity sensing, p53 activation, and inhibition of cell cycling. Our present work focuses on a more recently discovered PAH, dibenzo[*a,l*]-pyrene (DB[*a,l*]P or DBP also known as dibenzo[*def,p*]chrysene, DBC). This PAH is purportedly the most potent PAH carcinogen known. However, the immunotoxicity of DBP has not been characterized. Our work shows that DBP is, in fact, a potent mouse immunosuppressant that also appears to work via the formation of diol-epoxides and genotoxicity. Finally, recent studies in our lab have shown that arsenic may interact synergistically with PAHs to increase the amount of immunotoxicity. The mechanism may relate to inhibition of DNA repair mediated by enzymes expressing zinc finger domains. Both *in vitro* and *in vivo* studies show that no effect levels of PAH exposures in mice become significantly immunosuppressive when co-exposures occur with sodium arsenite. The results of these studies and future directions for this work will be discussed.

Scott W. Burchiel, Ph.D., (505) 272-0920, sburchiel@salud.unm.edu

TUNING OF STRESS RESPONSE BY GLUTATHIONE IN STEATOHEPATITIS

Chen Y[a], Singh S[a], Matsumoto A[a], Shertzer HG[b], Vasiliou V[a].
[a]: Department of Pharmaceutical Sciences, University of Colorado AMC, Aurora, CO 80045
[b]: Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH 45267

Steatohepatitis, featuring excessive fat accumulation and necroinflammation in the liver, may appear as the hepatic lesion in diseases of various etiologies, including metabolic disorders, alcohol consumption, viral hepatitis and drug overdose. Intrahepatocyte generation of oxidants is believed to be a critical mediator in the pathogenesis of these hepatopathies. Glutathione (GSH), the most abundant intracellular non-protein thiol, has several ascribed biological functions, most notably electrophile detoxication and oxidant elimination. Aside from acting as an antioxidant, GSH is increasingly recognized as a redox signaling molecule via post-translational modification of proteins. Recent studies using transgenic rodent models of GSH deficiency have shown a differential hepatic response to distinct profiles of hepatic GSH depletion. A dramatic GSH deficiency (5% of normal) accompanying mitochondrial failure leads to severe steatohepatitis and early mortality. At comparable level of GSH deficit, albeit with mitochondrial function partially preserved, moderate steatohepatitis develops and progresses to cirrhosis. On the contrary, in animals where hepatic GSH is less severe (15% of normal) and chronic, hepatic and mitochondrial functions remain intact; furthermore, these animals are resistant to ethanol-induced steatosis due to impaired lipid metabolism and exhibit beneficial stress response. Taken together, these results indicate a potential role of hepatic GSH pool in fine tuning of metabolic and stress response in liver injuries.

IMMUNOTOXICITY TESTING STRATEGIES IN PHARMACEUTICAL DRUG DEVELOPMENT

Mark Collinge, PhD
Principal Scientist, Immunotoxicology Center of Emphasis, Pfizer Worldwide Research and Development. Groton, Connecticut, 06340

The field of immunotoxicology encompasses a variety of sub-disciplines, including immunomodulation (both immunosuppression and immunoenhancement), hypersensitivity reactions, and immunogenicity testing/anti-drug antibodies. While hypersensitivity and immunogenicity will be briefly considered, the primary focus of this presentation will be on immunomodulation. Pharmaceuticals for various therapeutic indications may impact the immune system, either intentionally or unintentionally. In addition to expected effects on the immune system, unexpected immunotoxicity may be a consequence of off-target effects or exaggerated pharmacology. There is no standard battery of assays used for immunotoxicity testing, and the immunotoxicity testing strategies adopted for intentional vs. unintentional immunotoxicants will likely be quite different, and are developed on a case-by-case basis. This presentation will discuss the factors to consider in developing immunotoxicity testing strategies for pharmaceutical drugs. Discussion will include possible triggers for immunotoxicology testing, in addition to assays that may be used to attempt to de-risk immunomodulatory compounds. The timing of nonclinical immunotoxicity testing with respect to the drug development process will also be considered. Brief consideration will be given to the guidelines which govern immunotoxicology assessments, with particular emphasis on ICH S8. An overview of some of the current assays most frequently used (both for innate and adaptive immunity) in immunotoxicity assessments will be presented, along with a description of a recently developed human cell-based assay which can be used early in drug development to detect the potential for immunosuppression.
Mark Collinge, Ph.D., 860-686-3092

DISCOVERY & INVESTIGATIVE TOXICOLOGY: LEAD OPTIMIZATION & ISSUE INVESTIGATION TO ENABLE CANDIDATE IDENTIFICATION & RATIONALE DECISION-MAKING

Donna M. Dambach, VMD, PhD, DipACVP
Director, Safety and Investigative Toxicology,
Safety Assessment, Genentech, Inc.

Toxicity has been a significant cause of drug attrition driving the need to implement strategies and approaches to efficiently and effectively identify significant liabilities early in discovery and develop screening models to enable medicinal chemists to “dial-out” those liabilities to choose candidate compounds with the best safety profiles to go into humans. Engaging in issue investigation during this stage also helps to characterize the potential relevance of a finding to humans, minimize unanticipated toxicities during the time-constrained early development period, and allows for rationale, weight-of-evidence decision-making. Thus, the combination of discovery and development toxicology activities is complementary and enhances our ability lower attrition and inform drug design to improve safety profiles.

TREATMENT OF CHRONIC HEPATITIS C WITH FIRST GENERATION NS3/4A PROTEASE INHIBITORS

Gregory T. Everson, MD
Professor of Medicine, Director of Hepatology, University of Colorado Denver

Approximately 4 to 5 million Americans are infected with the hepatitis C virus (HCV). Chronic hepatitis C may progress to cirrhosis in up to 25% of infected individuals over the course of their lifetime and is a leading cause of hepatocellular cancer and the major indication for liver transplantation. The treatment of chronic hepatitis C has evolved over the last 20 years. HCV was initially treated with 6 to 12 months of standard interferon, but rates of sustained virologic response were only 5 to 10%. The addition of ribavirin (RBV) and pegylated interferon (PEGIFN) improved these results and until this year, 2011, this combination (PEGIFN+RBV) was the standard-of-care (SOC) for treatment of the chronic hepatitis C virus. However, despite the improvement, rates of sustained virologic response (SVR) for patients with HCV, genotype 1, were still less than 50%. Given that HCV genotype 1 represents 75% of HCV cases in the US, Europe, Japan, and China – additional therapies targeting the replication machinery of HCV were developed. Two new direct acting antivirals, Telaprevir and Boceprevir, were just recently approved this year for the treatment of chronic hepatitis C. The new emerging SOC, Triple Therapy, using Telaprevir or Boceprevir with PEGIFN/RBV promises to improve SVR for HCV genotype 1 infection to ~75%. Telaprevir and boceprevir are inhibitors of HCV NS3/4A serine protease an enzyme critical for viral replication. Although, Triple Therapy is more effective than PEGIFN/RBV, the treatment present new challenges. New side effects, such as skin rash, dysgeusia, anal pain, and anemia require close monitoring, assessment and unique management. The treating provider must effectively manage dosage and adherence to the regimen to avoid emergence of resistant variants of HCV. Close monitoring of patients, awareness of drug interactions, management of known telaprevir and boceprevir side effects, and strategies for reduction or discontinuation of individual drugs in the treatment regimen will be critical to ultimate success of Triple Therapy.

MATERNAL HIGH FAT DIET PROGRAMS FETAL HEPATIC LIPOTOXICITY AND EARLY METABOLIC DISEASE IN THE NON-HUMAN PRIMATE.

Jacob E. (Jed) Friedman
Professor of Pediatrics, Biochemistry & Molecular Genetics, University of Colorado Denver School of Medicine, Aurora, CO.

Extensive human epidemiologic and animal model data indicate that the maternal and early postnatal environment, especially nutrition, may permanently alter the infant's long-term risk for obesity and metabolic diseases by altering organ structure and function. Further, both nutrition and other environmental stimuli have been shown to alter gene expression through the induction of epigenetic changes such as DNA methylation and histone modification in cells and tissues. The nature of these events and how these molecular changes are translated into increased risk for obesity in the future remains unclear. Previously we reported that non-human primate fetuses (early 3rd trimester) exposed to a maternal high fat diet (HFD) had increased liver triglycerides, inflammation, and oxidative stress in utero, suggesting profound effects on fetal metabolism (JCI, 2009). We have extended these findings to offspring at 1 yr of age to determine the programming effects of maternal HFD exposure on the juvenile liver and the risk of obesity and diabetes. This lecture will discuss the evidence for fetal metabolic programming of adult disease focusing on the early origins of fatty liver, with an emphasis on inflammatory responses, glucose, and lipid metabolism in Non-Human primates. The potential for detrimental maternal lipid burden in the developing human fetal liver on metabolic complications of childhood obesity will also be discussed.

PROFILING PROTEIN CARBONYLATION IN A MURINE MODEL OF ALCOHOLIC LIVER DISEASE

James J. Galligan, Kristofer S. Fritz, Rebecca L. Smathers and Dennis R. Petersen

Department of Pharmaceutical Sciences, School of Pharmacy, The University of Colorado Denver, Aurora, CO.

Hepatic oxidative stress is a recognized result of sustained ethanol consumption. The resulting formation of lipid peroxidation end-products have been proposed to play a role in a host of disease pathologies related to sustained oxidative stress. These reactive electrophiles are well known to form covalent adducts with protein side-chains (e.g. protein carbonylation) resulting in altered structure, activity and localization. To investigate the role of protein carbonylation in alcoholic liver disease (ALD), a 6-week mouse model was employed. Ethanol-fed mice displayed a 2-fold increase in hepatic TBARS while immunohistochemical analysis for the reactive aldehydes 4-hydroxynenenal (4-HNE), 4-oxononenal (4-ONE) and malondialdehyde (MDA) revealed a significant increase in the staining of modified proteins. Increased protein carbonyl content was confirmed utilizing subcellular fractionation of liver homogenates followed by biotin-tagging through hydrazide chemistry, where approximately a 2-fold increase in modified proteins was observed in microsomal and cytosolic fractions. Novel targets of protein carbonylation were identified using a secondary hydrazide method coupled with 2-dimensional liquid chromatography tandem mass spectrometry (2D LC-MS/MS). Our results identify numerous protein targets for modification by 4-HNE, 4-ONE and MDA in cytosolic, nuclear, mitochondrial and microsomal fractions. The presence of novel *in vivo* sites of adduction by these aldehydes has also been confirmed in our model. Bioinformatic analyses were also conducted with protein identifications in the ethanol-fed mice. These analyses revealed 60 significant functional annotation clusters, outlining pathways associated with fatty acid and lipid metabolism, amino acid metabolism, chaperone activity, oxidoreductase activity and gluconeogenesis. Collectively, these data reveal pathway-specific effects of protein carbonylation in the pathogenesis of ALD.

James Galligan, 303-724-3398, James.Galligan@UCDenver.edu

INFLAMMATION, LIVER REGENERATION, AND LIVER CANCER: A KEY ROLE OF INTERLEUKIN-22

Bin Gao, MD., PhD

Laboratory of Liver Diseases, NIAAA, National Institutes of Health, Maryland, USA

Inflammation is known to be a key factor causing chronic liver injury, cirrhosis, and liver cancer. However, the underlying mechanisms remain largely unknown. Here we demonstrated that interleukin 22 (IL-22), which acts as either a pro-inflammatory or anti-inflammatory cytokine in various disease models, is markedly upregulated in chronic liver diseases, including viral hepatitis B and C infection. A strong correlation between IL-22 expression in the liver with active, inflammatory human liver disease was also found. To clarify the role of IL-22 upregulation in the pathogenesis of liver diseases, liver specific IL-22 transgenic (IL-22TG) mice, under the control of albumin promoter, were developed. Despite elevated IL-22 serum levels ranging from 4000 to 7000 pg/ml, IL-22TG mice developed normally without obvious adverse phenotypes or evidence of chronic inflammation. Most interestingly, IL-22 TG mice were completely resistant to Concanavalin A-induced T cell hepatitis with minimal effect on liver inflammation and had accelerated liver regeneration after partial hepatectomy. Although they did not spontaneously develop liver tumors, IL-22 TG mice were more susceptible to d*iethylnitrosamine*-induced liver cancer. Microarray analyses revealed that a variety of anti-oxidant, mitogenic, acute phase genes were upregulated in the livers from IL-22TG mice compared with those from wild-type mice. These findings indicate that localized production of IL-22 by inflammatory cells in the liver promotes hepatocyte survival and proliferation but primes the liver to be more susceptible to tumor development without significantly affecting liver inflammation. *This work was supported by NIAAA/NIH intramural funds*

ARSENIC-INDUCED AUTOPHAGY: LIFEBOAT OR TORPEDO?

Bolt A.M., Zhao F.,Klimecki W.T.
Department of Pharmacology and Toxicology, University of Arizona

Arsenic exposure in human populations has been definitively associated with a wide range of chronic diseases, both cancerous and non-cancerous. Despite this incontrovertible data, few arsenic-associated diseases have been assigned a clear, mechanistic explanation.A complete understanding of the cellular pathways activated by arsenic exposure is essential to understand the mechanism(s) underlying human diseases associated with arsenic exposure. Our studies of lymphoblastoid cell lines (LCL) exposed to arsenite at environmentally relevant levels (about 55 ppb) have shown up-regulation of autophagy to be a prominent feature of arsenite exposure, even at non-cytotoxic exposure levels. Evidence for activation of autophagy in LCL includes electron micrographic evidence of typical autophagosomes and their precursor structures. Additionally we observed an increase in the total cellular volume of acidic vesicles stained with an acidophilic, fluorescent dye, Lysotracker Red. Finally we measured an increase in the lysosome-dependent turnover rate of LC3-II protein, considered to be a specific marker for autophagy induction.In extended exposures to arsenite in culture (8-14 days), these changes were accompanied by a global, genome-wide induction in the expression of genes whose products localize to the lysosome, including several cathepsin family members. This induction was associated with increased levels of transcription factor EB (TFEB) a transcription factor capable of coordinate induction of lysosomal genes as well as induction of autophagy. Our work in this model has focused on the cellular targets of arsenite that initiates the autophagy process. Our studies in LCL suggests that arsenite induces endoplasmic reticulum stress as well as elements of the unfolded protein response (UPR), both processes that have been shown to be capable of inducing autophagy. Extending these studies to other cell types suggests that these cellular responses to arsenite exposure are not unique to LCL, but rather they are general responses observed in primary human cells, and in diverse malignant cell lines. Current work in our group is focusing on understanding the balance between the cell survival Vs. cell death effect of arsenite-induced autophagy, in light of research showing that autophagy has the capacity to induce both downstream consequences. (Supported by NIEHS ES006694, ES04940, ES16652)

Walter Klimecki: klimecki@pharmacy.arizona.edu, 520-626-7470

ANALYSIS OF ENDOPLASMIC RETICULUM (ER) STRESS-RELATED LIPOGENESIS GENES IN PROGRESSIVE HUMAN NONALCOHOLIC FATTY LIVER DISEASE

April D. Lake*, Petr Novak*†, Rhiannon N. Hardwick*, Brieanna Flores-Keown*, Nathan J. Cherrington*
*Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.
†Biology Centre ASCR, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic.

Human nonalcoholic fatty liver disease (NAFLD) is a chronic liver condition that is continually increasing in worldwide prevalence. NAFLD originates as steatosis, which may then progress to non-alcoholic steatohepatitis (NASH). NASH is characterized by an increase in oxidative stress, inflammation and fibrosis. The purpose of the current study was to confirm activation of ER stress signaling in human NAFLD liver samples, and investigate perturbations in ER stress-related lipogenesis. Signaling of the ER stress pathway activates the unfolded protein response (UPR) through three trans-membrane sensors including: inositol-requiring enzyme 1 (IRE1), PKR-like kinase (PERK), and activating transcription factor-6 (ATF6). The intermediary proteins and downstream transcription products of these pathways coordinate gene expression changes that reduce the occurrence of misfolded proteins in the ER through the UPR. An additional role of ER stress is the regulation of lipogenesis. Global ER stress and lipogenesis gene expression was analyzed using Affymetrix GeneChip Human 1.0ST arrays. Differential gene expression was analyzed between three clinically defined pathological groups including normal, steatosis and NASH. Genes designated as *de novo* lipogenesis genes were found to be preferentially down-regulated in NASH. Phosphorylated and total protein expression of ER stress intermediary proteins including eukaryotic initiation factor 2 alpha (eIF2alpha) and c-jun N-terminal kinase (JNK) were analyzed by immunoblot analysis. Phosphorylation of these two proteins was increased in NASH samples. Furthermore, X-box binding protein 1 (XBP-1), a critical lipogenesis and ER stress effector, exhibited an increase in nuclear localization in steatosis and NASH, indicating activation of the protein. Interestingly, XBP-1 mRNA showed a concomitant decrease with de novo lipogenesis genes. In summary, this work confirms the presence of the XBP-1 ER-stress mediated downregulation mechanism of de novo lipogenesis in human NASH.

April D. Lake, (520) 626-1531, lake@pharmacy.arizona.edu

COMPARATIVE BIORESPONSES OF QUANTUM DOT SIZE, CHARGE AND FUNCTIONALIZATION IN PRIMARY HUMAN LUNG EPITHELIAL CELLS.

Amber Nagy[a], Andrea Steinbruck[b], Jun Gao[a], Jennifer Hollingsworth[b] and Rashi Iyer[a] [a] BioScience Division, Los Alamos National Laboratory, [b]Center for Integrated Nanotechnologies, Los Alamos National Laboratory

Quantum dots (QDs) are semiconductor nanocrystals possessing unique optical properties and can be used for a wide variety of applications, from photovoltaics to biomedicine. The physicochemical properties of QDs, such as size, charge, functionalization, composition and redox activity all impact their interactions with biological systems and eventually dictate their utility. In our project we attempt to understand and correlate the effect of each of these individual properties to specific types of biological responses. Because one of the main mechanisms of exposure to nanomaterials is via inhalation, the current study focuses on the cellular responses of primary human bronchial epithelial cells (NHBE) after exposure to QDs. Specifically, we have tested NHBE reactive oxygen species (ROS) formation, apoptosis, necrosis and proliferation in response to CdSe-based QDs. To further understand how QD size, charge and functionalization impact NHBE cell responses, we have modified 3, 5 and 10 nm CdSe QD to carry a positive or negative charge by capping QDs with four different ligands that differ in length. Mercaptopropanoic acid (MPA) and cysteamine (Cyst.) served as our short caps, and provided QDs with negative and positive charges, respectively. Mercaptoundecanoic acid (MUA) and aminoundecanethiol (AUT) served as our long ligands, where the former gave QDs a negative charge and the latter provided QDs with a positive charge.
Our results revealed that positive QDs are far more toxic to NHBE cells compared to negative QDs, at far lower concentrations. While the primary mechanism of QD toxicity is necrosis, CdSe QDs capped with cysteamine were found to induce apoptosis in a dose-dependent manner. Accordingly, a decline in proliferation was noted in NHBE cells that had been exposed to QDs capped with long ligands. We also determined that positive and negative QDs capped with longer ligands induce more ROS formation, and that these levels correlate with QD size for negatively charged QDs. Dynamic light scattering data revealed that QDs capped with long ligands form smaller aggregates in biological media, which may be contributing factor to their toxicity.
In summary, we have found that QD surface charge appears to be a deciding factor in the ability of QDs to induce cellular level toxic response. In addition, we have found that ligand length also plays a role in the mechanism of toxicity and should also be considered an important factor that can influence cellular responses to nanomaterials.
Amber Nagy, email: anagy@lanl.gov, phone: (505)-665-7850

ACTIVATION OF TRPA1, V1, AND M8 BY COMBUSTION-DERIVED PARTICULATE MATERIALS: RELATIONSHIP TO LUNG INFLAMMATION AND INJURY

Christopher A. Reilly
Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

Transient receptor potential (TRP) calcium channels regulate many adverse physiological responses to diverse environmental stimuli. TRPA1, V1, and M8 have been identified as proximal sensors of two unique combustion-derived environmental particulate pollutants (PM): diesel exhaust PM (DEP) and coal fly ash PM (CFA), and to mediate key elements of pneumotoxicity by these materials. TRPA1 was activated by DEP, and by specific chemical entities on DEP, via covalent modification of the TRPA1 electrophile/oxidant sensitive site. Inhibition of DEP-induced calcium flux in cultured sensory neurons by HC-030031 confirmed TRPA1 as the major sensor of DEP, and a probable pathway for DEP-induced airway reflex responses and inflammation. Conversely, TRPV1 and M8 were minimally activated by DEP, and DEP-associated TRPA1 agonists, but were activated by two different form of CFA. TRPV1 activation occurred via direct contact between cell-surface amino acids and the insoluble, mineral core components of CFA, which also activated TRPA1, the TRPA1-3CK (electrophile insensitive) mutant, and TRPM8, suggesting common mechanosensory activities for all three TRP channels. Cell surface TRPV1 was confirmed as the principal mediator of CFA-induced IL-6 and 8 expression by cultured human lung bronchial epithelial cells, which lack TRPA1 and TRPM8 expression, as well as a key determinant of cytokine/chemokine induction in mouse lungs, where 40-60% inhibition was observed in TRPV1-/- mice. These studies partially elucidate the molecular and chemical basis for differential detection and responses to PM in the respiratory tract by illustrating that different PM activate different cell-specific processes via distinct mechanisms, supporting the idea that TRP channels may be selectively targeted to limit air pollution toxicity. Support: ES017431.

Dr. Christopher A. Reilly, Ph.D.,801-581-5236, Chris.Reilly@pharm.utah.edu

PHARMACEUTICAL TOXICOLOGY - INTEGRATION OF REGULATORY REQUIREMENTS, MECHANISTIC INVESTIGATIONS AND RISK ASSESSMENT

T.P. Reilly, Drug Safety Evaluation, Research & Development, Bristol-Myers Squibb, Princeton, New Jersey

Nonclinical safety evaluation plays a critical role in the discovery, development and ultimately marketability of new pharmaceutical agents. The core mission of pharmaceutical toxicology organizations is to identify, characterize, resolve, and communicate nonclinical safety concerns of new drugs to inform scientifically sound and rationale assessments for humans. In silico, in vitro and in vivo experimental platforms are utilized to evaluate both target-mediated liabilities and off-target-related toxicity risks; to establish the dose- and exposure-response relationship for any effects; to determine their progression or regression over time; and to assess their ability to be clinically monitored. The timing and general requirements for these evaluations have been established by the International Committee on the Harmonization Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), but these guidelines do not exempt toxicologists from scientific considerations and strategy that may be specific to a particular molecule, disease target or indication. The ability to properly integrate findings, provide appropriate context, and decide when and how to interrogate critical mechanistic questions is both an expectation and a potential competitive advantage. Examples will be presented to illustrate the pivotal role that nonclinical toxicology plays in assessing the risk/benefit equation throughout the drug development process with the ultimate goal of supporting the safe and effective delivery of innovative medicines that help patients prevail over serious diseases.

ACTIVATION OF THE IRRITANT RECEPTOR TRPA1 BY WOOD SMOKE PARTICULATE MATERIAL AND ASSOCIATED CHEMICAL COMPONENTS.

Darien Shapiro, Cassandra E. Deering-Rice, Nick Hallstrom, Erin G. Romero, Garold S. Yost, John M. Veranth, and Christopher A. Reilly
Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Inhalation of combustion derived particulate matter (cdPM) in polluted air is correlated with many adverse health effects in humans. Wood smoke particulate matter (WSPM) is a common form of cdPM that is both extremely harmful and environmentally relevant across the globe. While it is clear that WSPM possesses a threat to human health, the molecular basis through which it causes adverse effects is not well defined. The hypothesis of this study was that TRP channels are activated by WSPM through direct binding of receptor active sites, electrophilic modification, and/or mechanical contact, as a mechanistic basis for pulmonary inflammation, injury, and ultimately respiratory dysfunction. Pine and mesquite PM were generated in the laboratory. These PM robustly activated TRPA1, but did not activate TRPV1, TRPV4, or TRPM8. Several WSPM components were also screened, revealing new TRPA1 agonists. Differential activation of TRPA1, as a function of WSPM particle size, demonstrated that the most potent WSPM were 2.5-10   m. The mechanism of TRPA1 activation by WSPM appeared to involve electrophilic modification of the electrophile/oxidant sensing domain of TRPA1 (i.e., the 3CK mutant is not activated), but ongoing studies are also evaluating the menthol binding site of TRPA1 as a target for WSP components based on findings that the TRPA1-3CK mutant may have limited function and that some WSP components are not electrophilic. This study identifies TRPA1 as a molecular sensor for WSPM which is hypothesized to be a critical step in elucidating the precise mechanisms by which WSPM causes toxicity in the respiratory tract. Support: NIH grant ES017431, The University of Utah Biology Undergraduate (BioURP) program, and the Aslam Foundation/Juan Diego Catholic High School Summer research program.

ARSENIC ALTERS BARRIER PROPERTIES IN AIRWAY EPITHELIAL CELLS

C.L. Sherwood[1,2,5], A.E. Liguori[1,5], C.E. Olsen[1], B. Barcelo[1,3,5], J.L. Burgess[4,6], R.C. Lantz[2,4,5], and S. Boitano[1-5]
[1]Arizona Respiratory Center, Departments of [2]Cell Biology and Anatomy, and [3]Physiology Arizona Health Sciences Center; [4]Southwest Environmental Health Sciences Center, [5]Bio5 Collaborative Research Institute, and [6]Mel & Enid Zuckerman College of Public Health, University of Arizona

As a key role in innate immune function airway epithelial cells provide a barrier that protects underlying tissue from inhaled particulates and toxicants frequently found in inspired air. Three categories of membrane spanning tight junction (TJ) proteins contribute to barrier formation: the junction adhesion molecules, the claudins, and occludin. Arsenic is a unique lung toxicant in that it can lead to respiratory illness through both inhalation and ingestion, most commonly through contaminated drinking water. In animal and human studies, we and others have shown that arsenic ingestion can lead to altered lung function suggestive of epithelial barrier dysfunction. In this report, we evaluated the effects of environmentally relevant levels of arsenic (i.e., < 4 M (~300 g/L) as Na-arsenite) on airway epithelial barrier properties. In our primary mouse tracheal epithelial (MTE) cell model we found that both micromolar (3.9 M) and sub-micromolar (0.8 M) arsenic concentrations reduced transepithelial resistance, a measure of TJ function. Immunofluorescent staining showed altered localization of claudins -1, -4, and occludin in arsenic treated MTE cells. In order to better quantify changes in tight junction molecular components induced by arsenic we used an immortalized human bronchial epithelial cell line (16HBE14o-). We found that micromolar and submicromolar concentrations of arsenic increased the protein expression of claudins -4, -5, and -7 as well as the mRNA expression of claudin-7 in 16HBE14o- cells. Additionally, micromolar levels of arsenic resulted in altered phosphorylation of occludin. In summary, exposure to environmentally relevant levels of arsenic can alter both the structure and function of airway epithelial TJs, and consequently, basic innate immune defense in the airway.

Cara Sherwood, (520) 906-3044, caras@email.arizona.edu

THE ROLE OF ALDH1B1 IN ALCOHOL METABOLISM, COLON CARCINOGENESIS AND DIABETES: CHARACTERIZATION OF *Aldh1b1* NULL MICE

Singh S.[1], Chen Y.[1], Matsumoto A.[1], Gavalas A.[2], and Vasiliou V.[1]
[1]Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045
[2]Stem Cell Developmental Biology Laboratory
Biomedical Research Foundation of the Academy of Athens, Athens 11527

Aldehyde dehydrogenases (ALDHs) are a group of NAD(P)+-dependent enzymes involved in the metabolism of a wide variety of aliphatic and aromatic aldehydes. ALDH1B1 is a mitochondrial homotetrameric enzyme with a subunit of 517 amino acids that is 65% and 72% identical to ALDH1A1 and ALDH2 proteins, respectively. ALDH1B1 usesNAD$^+$ as the cofactor and exhibits high affinity for aliphatic aldehydes, especially acetaldehyde, indicating an important role of ALDH1B1 in ethanol metabolism. Recent studies have provided new evidence supporting novel physiological functions of ALDH1B1. Our recent studies have shown that ALDH1B1 is a potential biomarker for human colon adenocarcinoma, and this enzyme may be involved in pancreas development. To assess the *in vivo*role of ALDH1B1,we have developed a transgenic*Aldh1b1(-/-)* null mouse line.These knockout mice have a normal growth pattern and are fertile.The ALDH1B1 mRNA and protein are absent in various organs of *Aldh1b1 (-/-)* mice. When compared to *Aldh1b1(+/+)* littermates, *Aldh1b1(-/-)* mice have higher fasting blood glucose levels and show a trend of decreased glucose tolerance and have decreased insulin signal in pancreas. Alcohol pharmacokinetics and experimental colon carcinogenesis using *Aldh1b1(-/-)* and *Aldh1b1(+/+)* mice are currently in progress. Collectively these data suggest a role of ALDH1B1 in alcohol metabolism, colon carcinogenesis and pancreatic development.Future studies are warranted to elucidatethe mechanistic role of ALDH1B1 in these processes.
Surendra Singh,303-724-3521, Surendra.singh@ucdenver.edu

4-HNE SIGNIFICATLY ALTERS L-FABP STRUCTURAL AND FUNCTIONAL DYNAMICS

RL Smathers, P Reigan,KS Fritz, JJ Galligan, CT Shearn and DR Petersen
Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

Lipid peroxidation (LPO) is implicated in the pathogenesis of various diseases involved with chronic oxidative stress–including diabetes, Alzheimer's, Parkinson's, Non-Alcoholic Fatty Liver Diseaseand Alcoholic Liver Disease (ALD). Reactive aldehydes generated from LPO, such as 4-hydroxynonenal (4-HNE), often result in protein modification through adduction of protein side chains. The specificity of these modificationsis protein-dependent,frequentlyposingdetriment to conformational structure, stability and function. Utilizing a proteomic wide scan for 4–HNE modified proteins, liver fatty acid binding protein (L-FABP) was found in hepatic cytosolic fractions from both rats and mice chronically fed an ethanol-containing diet. Sites of modification were identified byMALDI-TOF/TOF mass spectrometry in both the lipid-bound (holo) and unbound (apo) states of recombinant mouse L-FABP *in vitro.* Two adducts (Lys57 andCys69) were found on the periphery of apo-L-FABP, while six peripheral adducts (Lys6, Lys31, His 43, Lys46, Lys57 and Cys69) were found on holo-L-FABP.Molecular modeling, in conjunction with binding and stability assays, has provided novel insights into the consequences of L-FABP adduction by 4-HNE. These modificationswere found to significantly reduce the area, shape and structural integrity of the binding pocket and ligand portals. Furthermore, a fourth, solvent accessible, binding portal for ligand entry/exit in holo L-FABP was identified post-adduction. The dynamics of these modeling simulations is supported with *in vitro* data, showing decreased stability of adducted protein in the apo state and decreased capacity and affinity for multiple fatty acids. Collectively, these data demonstrate the consequences of L-FABP adduction by 4-HNE*in silico*and *in vitro* – revealing insight into the complex molecular dynamics involved in protein modification via reactive aldehydes. (Supported by R37 NIH/AA009300 (DRP) and NIH/AAA F31 AA18898-03 (RLS).

Contact: Rebecca Smathers, 303-724-3398, Rebecca.Smathers@ucdenver.edu

GENE DELETION OF NOS2 PREVENTS MANGANESE-INDUCED NEUROLOGICAL INFLAMMATION IN DEVELOPING MICE

Karin M. Streifel, Julie A. Moreno, Marie Legare, and Ronald B. Tjalkens. *Center for Environmental Medicine, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA*

Overexposure to the essential nutrient manganese (Mn) can lead to a degenerative neurological disorder termed manganism. Reactive gliosis and subsequent neuroinflammation are implicated in the progression of the disease, with selective increases in reactive oxygen and nitrogen species associated with neuronal injury. Previous studies from our laboratory demonstrated that expression of inducible Nitric Oxide Synthase (iNOS/NOS2) is increased in astrocytes in mice sub-chronically exposed to Mn and that this increase associates with elevated levels of 3-nitrotyrosine protein adducts in neurons, a marker of nitrosative stress. Based upon these data, we postulated that mice lacking NOS2 would be protected against the neurotoxic effects of Mn*in vivo* and *in vitro*. Juvenile mice were exposed to 50 mg/kg of $MnCl_2$ by intragastric gavage. Our results indicate protection of locomotor deficits in $NOS2^{-/-}$ mice, histopathological activation of astrocytes and microglia from Mn-treated mice in both genotypes, but a decrease of 3-nitrotyrosine adduction on neurons in the basal ganglia in the $NOS2^{-/-}$Mn-treated mice correlating inflammatory neurotoxicity with the Mn-induced motor defects. In addition astrocyte cultures treated with $MnCl_2$ and cytokines produced apoptotic caspase activation in wild type striatal neurons, but these markers were attenuated with treatment upon $NOS2^{-/-}$ astrocytes. These data demonstrate that lack of $NOS2^{-/-}$ plays a crucial role in preventing the activation of the Mn-induced inflammatory phenotypes *in vivo* and suggests that further suppression of this gene could be a valuable target for Mn-toxicity treatment.

DRUG METABOLISM AND IDENTIFYING COMPOUND LIABILITY AND ITS IMPACT IN DRUG DEVELOPMENT

Michael F. Wempe, PhD.
Associate Research Professor, Department of Pharmaceutical Sciences and Director, Medicinal Chemistry Core Facility, University of Colorado Denver Anschutz Medical Campus

Abstract: The development of new drugs is expensive, time intensive, and packed not only with scientific issues but heavily influenced by business politics which may appear counter intuitive to the philanthropic concept of actually helping people (*i.e.* poor marketing projections / low profit margins). Furthermore, "many lead discovery candidates are transferred to the preclinical development process with insufficient characterization to assess their development potential. This lack of knowledge usually results in poorly designed experiments that are not data productive and that, in many cases, have to be repeated when a candidate shows unexpected toxicity, low and variable delivery, instability in formulation, or unacceptable pharmacokinetic and drug metabolism profiles. In … many cases, these problem areas [result] in termination of development for a potentially useful therapeutic agent." New Drug Approval Process (3rd Edition; page 33-34). In this talk – keeping in mind our limited time – various scientific issues related to the pre-clinical development of a novel oncology drug will be presented. The goal is to provide an example (*i.e.* case study) to illustrate and high-light various compound liabilities that one should think about during the development process.

THERAPEUTIC POTENTIAL OF NRF2 ACTIVATORS IN STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY

Samantha A Whitman[1], Hongting Zheng[1,2,], Wei Wu[1,2], Georg Wondrak[1], Pak K. Wong[3], Deyu Fang[4], and Donna D. Zhang[1]
[1]Department of Pharmacology and Toxicology, University of Arizona, Tucson, Arizona, [2]Department of Endocrinology, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, PR China,

[3]Department of Aerospace-Mechanical Engineering, University of Arizona, Tucson, Arizona, [4]Department of Pathology, Northwestern University, School of Medicine, Chicago, Illinois.
ABSTRACT: OBJECTIVE—To determine whether dietary compounds targeting Nrf2 activation can be used to attenuate renal damage and preserve renal function during the course of STZ-induced diabetic nephropathy (DN).
RESEARCH DESIGN AND METHODS—Diabetes was induced in $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice by STZ injection. Nrf2 activators, Sulforaphane (SF) or cinnamic aldehyde (CA) was administered 2 weeks after STZ injection and metabolic indices and renal structure and function were assessed (18 weeks). Markers of diabetes including blood glucose, insulin, polydipsia, polyuria, and weight loss were measured. Pathological alterations and oxidative damage in glomeruli were also determined. Changes in protein expression of the Nrf2 pathway, as well as transforming growth factor beta 1 (TGF-β1), fibronectin (FN), Collagen IV, and p21/WAF1Cip1 (p21) were analyzed. The molecular mechanisms of Nrf2 mediated protection were also investigated in an *in vitro* model using human renal mesangial cells (HRMCs).
RESULTS—SF or CA significantly attenuated common metabolic disorder symptoms associated with diabetes in $Nrf2^{+/+}$ but not in $Nrf2^{-/-}$ mice, indicating SF and CA function through specific activation of the Nrf2 pathway. Furthermore, SF or CA improved renal performance and minimized pathological alterations in the glomerulus of STZ-$Nrf2^{+/+}$ mice. Nrf2 activation reduced oxidative damage and suppressed the expression of TGF-β1, extracellular matrix (ECM) proteins and p21 both *in vivo* and in HRMCs. In addition, Nrf2 activation reverted p21-mediated growth inhibition and hypertrophy of HRMCs under hyperglycemic conditions.
CONCLUSIONS—Experimental evidence is provided indicating that dietary compounds targeting Nrf2 activation can be used therapeutically to improve metabolic disorder and relieve renal damage induced by diabetes.

ARSENITE INTERACTS SELECTIVELY WITH ZINC FINGER PROTEINS CONTAINING C3H1 OR C4 MOTIFS

Xixi Zhou, Xi Sun, Karen L. Cooper, Feng Wang, KeJian Liu, Laurie G. Hudson

Department of Pharmaceutical Sciences, University of New Mexico,Albuquerque, NM 87131

Arsenic inhibits DNA repair and enhances thegenotoxicity of DNA damaging agents such asbenzo[a]pyrene and ultraviolet radiation.Arsenic interaction with DNA repair proteinscontaining functional zinc finger (zf) motifs isone proposed mechanism to account for theseobservations. Here we report that arsenite(As(III)) binds to both CCHC DNA bindingzinc fingers of the DNA repair proteinpoly(ADP-ribose) polymerase-1 (PARP-1).Furthermore, trivalent arsenite coordinateswith all three cysteine residues as demonstratedby tandem mass spectroscopy. MALDI-TOFMS analysis of peptides harboring site directedsubstitutions of cysteine with histidine residueswithin the PARP-1 zf revealed that arsenitebound to peptides containing three or fourcysteine residues, but not to peptides with twocysteines, demonstrating arsenite bindingselectivity. This finding was not unique toPARP-1; arsenite did not bind to a peptiderepresenting the CCHH zf of the DNA repairprotein aprataxin, but did bind to an aprataxinpeptide mutated to a CCHC zf. To investigatethe impact of arsenite on PARP-1 zinc fingerfunction, we measured the zinc content andDNA binding capacity of PARP-1immunoprecipitated from arsenite-exposedcells. PARP-1 zinc content and DNA bindingwere decreased 76% and 65%, respectively,compared to protein isolated from untreatedcells. We observed comparable decrease in zinccontent for xerodermapigmentosum group A(XPA) protein (CCCC zf), but not SpecificityProtein (SP) 1 or aprataxin (CCHH zf). Thesefindings demonstrate that PARP-1 is a directmolecular target of arsenite and that arseniteinteracts selectively with zinc finger motifscontaining ≥3 cysteine residues.

KeJian Liu, PhD, 505-272-9546; kliu@salud.unm.edu

ARSENITE INDUCES ENDOPLASMIC RETICULUM STRESS AND THE UNFOLDED PROTEIN RESPONSE IN LYMPHOBLASTOID CELLS

Fei Zhao*, Sandeep Anne*, Alicia M. Bolt*, Walter T. Klimecki*
*University of Arizona, Department of Pharmacology and Toxicology

Arsenic is a natural element ubiquitous in the environment. Long-term exposure to environmental inorganic arsenic has been associated with increased incidence ofmany complex diseases. The immune system is one target of arsenic. Epidemiological studies have pointed to the potential for arsenic to be an immunosuppressant. In vitro and ex vivo studies have demonstrated that arsenic impacts several elements of the immune response, including phagocytosis, cytokine release and an inhibition of lymphocyte proliferation. Previously, we reported that arsenite induces autophagy in human B lymphoblastoid cell lines (LCL). Autophagy is a cellular protective mechanism that functions in thedegradation of damaged cellular components, including protein aggregates formed by mis-folded or damaged proteins. This led us to consider whether the induction of autophagy by arsenite could be due to elevated endoplasmic reticulum (ER) stress and the corresponding unfolded protein response (UPR). Interestingly we have found that exposure of LCL to 1.5uM sodium arsenite induces the UPR downstream genes CHOP and GRP78 protein levels in lymphoblastoid cells. Correlated to the induction of CHOP and GFP78, there was also an increase in phosphorylated eIF2a and XBP1s protein level. Corresponding to the increase of phos-eIF2a, we found an inhibition of de novo protein synthesis rate in the arsenite treated lymphoblastoid cells. Corresponding to the increase of XBP1s, we found that XBP1 knockout mEF cells are more sensitive than wildtype to arsenite cytotoxicity.Together, our data suggests that ATO induces ER stress and the UPR in lymphoblastoid cells through the PERK/eIF2a and IRE1/XBP1 pathway.
Fei Zhao, (520) 626-2598, zhao@pharmacy.arizona.edu

HEPATOCYTE-SPECIFIC PROTECTION BY A2B ADENOSINE RECEPTORS DURING ISCHEMIA- REPERFUSION INJURY OF THE LIVER

Michael A. Zimmerman, MD,* Almut Grenz, MD, PhD, † Eunyoung Tak, PhD,† Maria Kaplan, BS,* Igal Kam, MD,* and Holger Eltzschig, MD, PhD†.
Division of Transplant Surgery*, and Department of Anesthesiology,† University of Colorado, Denver

Background. Ischemia-reperfusion injury (IRI) of the liver is among the leading causes of early hepatic dysfunction during liver transplantation. Recent studies have implicated extracellular adenosine production in liver protection from ischemia. At present, four distinct receptors for extracellular adenosine signaling have been characterized [A1 adenosine receptor [A1AR, A2AAR, A2BAR, A3AR]. Here, we hypothesized a contribution of AR signaling to liver protection from ischemia.
Methods. We used a hanging weight system to induce partial hepatic ischemia. Endpoints of IRI included liver transaminases, pro-inflammatory cytokines, myeloperoxidase ELISA for determination of neutrophil infiltration and histologic injury following 45 minutes of ischemia and 2 hours of reperfusion.
Results. In an initial screening experiment, we exposed gene-targeted mice for each individual AR to hepatic IRI. Surprisingly, we observed that genetic deletion of the A2BAR was specifically associated with worsened IRI. Moreover, treatment of wild-type mice with an A2BAR-selective antagonist resulted in enhanced liver injury, while an A2BAR agonist attenuated hepatic injury in wild-type mice, but not in *A2BAR*$^{-/-}$ mice. To define the tissue-specific source of A2BAR-dependent liver protection during IRI, we generated novel mouse lines with tissue-specific deletion of the A2BAR on hepatocytes or vascular endothelia utilizing a transgenic mouse line with a floxed A2BAR. In fact, hepatocellular deletion of the A2BAR was associated with markedly increased hepatocellular injury, elevation of transaminases and pro-inflammatory cytokines, while mice with endothelial A2BAR deletion were not different from wild-type animals.
Conclusions. These studies reveal a novel contribution of hepatocyte-specific adenosine signaling via the A2BAR to liver protection from ischemia. As such, these studies point towards a potential role for A2BAR agonists to improve early graft function following liver transplantation.

Poster Abstracts

POSTER # 1

TOXIC GLUCOSINOLATE PRODUCTS FROM THE INVASIVE PLANT *CHORISPORA TENELLA*

Olivia R. Arnold and Howard S. Ramsdell.
Colorado State Univeristy/Center for Environmental Medicine

Chorispora tenella is an introduced species in North America and has been described in at least 31 states, including all of those west of the Mississippi, and 3 Canadian provinces. It is known by several common
names, including blue mustard, crossflower and purple mustard. It is among the brassica species that have been blamed for causing toxic effects in animals. Toxicity of plants in the mustard family is generally attributed to the products of enzymatic hydrolysis of glucosinolates. As little
phytochemical information is available for C. tenella, we have investigated potentially toxic constituents of the plant. Samples were collected in the spring in the vicinity of Fort Collins, Colorado. They were chilled immediately and kept at 4°C until analysis. Freshly minced leaves were placed in a vial, covered with water and immediately capped. When subjected to headspace gas chromatography-mass spectrometry analysis, allyl isothiocyanate (AITC) was the only compound detected. Plant samples were homogenized in pH 7.5 citrate/phosphate buffer and incubated at room temperature in a sealed flask with an overlying layer of pentane to extract volatile products. This autolysis process would be akin to what would happen should a grazing animal eat the plant. Analysis of the resulting extracts by GC-MS showed recovery of 35.3 +/- 7.6 micrograms of AITC per gram of plant fresh weight (mean +/- sd, n = 5). Other compounds
detected at substantially lower levels included propyl isothiocyanate and butyl isothiocyanate. Liver, kidney and thyroid toxicity have been associated with AITC exposure. Other products of C. tenella autolysis may also contribute to adverse effects if the plant is consumed by animals.

Olivia Arnold, 818-679-1737, Olivia.arnold@colostate.edu

POSTER #2

NANOPARTICLE TOXICITY ON AIRWAY EPITHELIAL CELLS

Scott Boitano[1,-5,7], Mia McCorkel[1,4,5], Cara L. Sherwood[1,2,4], Reyes Sierra-Alvarez[6], James A. Field[6,7], and Farhang Shadman[6]
[1]Arizona Respiratory Center, Departments of [2]Cell Biology and Anatomy, and [3]Physiology, Arizona Health Sciences Center; [4]Bio5 Collaborative Research Institute, [5]Biomedical Engineering Graduate Interdisciplinary Program, [6]Department of Chemical and Environmental Engineering, and [7]Southwest Environmental Health Sciences Center, University of Arizona.

There are strong correlations between ultrafine particle (i.e. nanoparticle) deposition in the lung and chronic respiratory illness. The emergence of engineered nanoparticles (ENPs) presents a risk for lung exposure with potential adverse health effects. Recent research on chronic obstructive pulmonary disease (COPD) associated with ultrafine particle deposition has uncovered a key role for dysregulation of paracrine ATP signaling and corresponding innate immune function. We hypothesized that airway ENP exposure may affect cellular ATP signaling important in immune defense. We evaluated ATP signaling and its physiological impact on cultured human airway epithelial cells (16HBE14o-) following exposure to ENP hafnium oxide (HfO_2). Airway epithelial cells displayed a reduction in signaling and physiologic response to ATP following 24 hr exposure to low dose (10 - 250 mg/L) HfO_2. These results suggest that low doses of HfO_2 alter cellular signaling important in airway immune defense that could contribute to respiratory disease.

Scott Boitano, Ph.D. (520) 626-2105

POSTER # 3

ARSENITE INDUCES AUTOPHAGY IN RESPONSE TO PROTEIN AGGREGATION IN A GFP TRANSDUCED CELL LINE MODEL.

Alicia M. Bolt, Sandeep Anne, Fei Zhao and Walter T. Klimecki
Department of Pharmacology and Toxicology, University of Arizona, Tucson 85721

Arsenic is an metal found ubiquitously in the environment, contaminating water and food sources. Chronic arsenic exposure has been associated with a variety of diseases including cancer, neurodegenerative, dermatological, cardiovascular diseases, and immunosuppression. Previously, we have identified that arsenite-induced proliferative inhibition in human B lymphoblastoid cell lines (LCL) is associated with autophagy induction. Autophagy is an inducible cellular compensatory response to environmental toxicants and stress. Autophagic pathways organize the removal of improperly folded proteins and damaged organelles by lysosomal degradation. To pursue our observations that arsenite induces endoplasmic reticulum stress and the unfolded protein response in LCL, we stably transduced LCL with green fluorescent protein (GFP) a long-lived protein that has been shown to be capable of forming aggregates. An 8-day arsenite exposure (1.5 uM) to GFP-transduced LCL (GFP-LCL) resulted in the induction of autophagy characterized by 1) inhibition of cellular growth, 2) expansion of acidic vesicles, 3) increase in the autophagosome marker, LC3-II and 4) substantial induction in transcription factor EB (TFEB) protein and mRNA levels. TFEB is a transcription factor that regulates the coordinated expression of lysosomal and autophagy related genes. Arsenite-induced autophagy in LCL was associated with an accumulation of GFP in lysosomes. Concomitant with this was the lysosomal accumulation of p62 and ubiquitin, suggesting that aggregated, improperly folded, or damaged proteins are delivered to the lysosome for degradation in response to arsenic exposure. Fluorescence microscopy image revealed that after arsenite exposure, GFP accumulates in large protein aggregates. This effect of arsenite in LCL is not unique to LCL, as we have reproduced these changes in other cell types, including the human embryonic kidney (HEK) 293 cell line. These data support the hypothesis that arsenite-induced autophagy is in response to the formation of protein aggregates, likely due to improperly folded proteins. GFP-transduced stable cell lines are a promising model for the investigation of toxicant-induced protein aggregation and its downstream consequences. (Funded by ES 04940, ES 006694, and DGE 0654435)
Alicia Bolt, 520-626-2598, bolt@pharmacy.arizona.edu

POSTER # 4

A LC-MS-MS METHOD FOR THE QUANTIFICATION OF PROSTAGLANDIN E2, D2, H2 AND F2 PRODUCTION IN CELL CULTURE SUPERNATANTS AND SUPPRESSION OF PROSTAGLANDIN PRODUCTION IN MURINE MACROPHAGES VIA LPS INDUCTION BY NOVEL COMPOUNDS: TOLFENAMIC ACID, 2,2'-DIINDOLYMETHANE, 5-DIINDOLYMETHANE AND 12-DIINDOLYMETHANE

Jennifer L. Berkbigler, Greg P. Dooley, Ryley A. McPeters, Maryam F. Afzali, William H. Hanneman

Center for Environmental Medicine, Department of Environmental Radiological and Health Sciences, Colorado State University, Fort Collins, CO 80523

Chronic inflammation has been implicated in the development of a number of human cancers, prostaglandin production is caused by such inflammatory mediators. Here we report an LC-MS-MS method that was developed and validated for the extraction and quantitation of prostaglandin E_2, D_2, H_2 and F_2 in cell culture supernatants. Prostaglandins were extracted using Isolute uncapped C18 solid phase cartridges, while UPLC and positive electrospray ionization was used with MRM tandem mass spectrometry for identification and quantitation. To account for differential loss and degradation of the analytes, isotope dilutions with deuterated standards were used for each analyte. The extraction method was validated with matrix matched spiked samples at the limits of quantitation for each analyte with recoveries ranging from 83%-105%. As an application for this method, RAW 264.7 murine macrophages were stimulated with 1ug/ml LPS. Novel 2,2'-Diindolymethane (DIM), 5-Diindolymethane, 12-Diindolymethane and tolfenamic acid were evaluated for their anti-inflammatory effects as measured by prostaglandin products. DIM compounds and tolfenamic acid were found to decrease the production of prostaglandin D_2 induced by LPS.

Jennifer Berkbigler,970-491-5128, jberkbig@rams.colostate.edu

POSTER # 5

ALTERED ARSENICAL DISPOSITION IN EXPERIMENTAL NONALCOHOLIC FATTY LIVER DISEASE

Mark J. Canet[*], Rhiannon N. Hardwick[*], April D. Lake[*], Michael J. Kopplin[†], A. Jay Gandolfi[*], Nathan J. Cherrington[*]

[*]University of Arizona, Department of Pharmacology and Toxicology, [†]University of Arizona, Center for Toxicology

Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in Western society. NAFLD represents a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which is characterized by an increase in inflammation and fibrosis within the liver. Liver damage sustained in the progressive stages of NAFLD may alter the ability of the liver to properly metabolize and eliminate xenobiotics. The purpose of the current study was to determine whether NAFLD alters the ability to metabolize and eliminate the environmental toxicant arsenic. Male C57bl/6 mice were placed on either a high fat or methionine and choline deficient diet to model simple steatosis and NASH, respectively. At the conclusion of the dietary regimen, all mice were given a single, oral dose of either sodium arsenate or arsenic trioxide. Mice with NASH excreted significantly higher levels of arsenic in urine (24h) compared to controls within. Total arsenic in liver and kidney of NASH mice was not different from control, however both tissues displayed differential retention of monomethyl (MMA) and dimethyl (DMA) arsenic metabolites. NASH livers retained significantly higher levels of MMA, whereas DMA is retained significantly less in the kidneys of NASH mice. Interestingly, urinary elimination of the more toxic trivalent inorganic arsenic species (iAs^{III}) was higher in mice with NASH compared to control along with preferential retention of pentavalent inorganic arsenic (iAs^{V}) in NASH livers. No change in the protein expression of hepatic arsenic (+3 oxidation state) methyltransferase was detected in NASH. Furthermore, protein expression of MRP1, a membrane transporter known to transport trivalent inorganic arsenic species, increased in the livers of NASH mice. These results suggest that NASH alters the normal disposition of arsenical species, and implicates cellular transport rather than biotransformation as a possible mechanism.

Mark Canet, (520) 626-1531, canet@pharmacy.arizona.edu

POSTER #6

HYPOTHIOCYANOUS ACID: CONSIDERATIONS OF CYTOTOXICITY AND REACTION WITH CRITICAL THIOLS

Joshua Chandler[1,2], Elysia Min[2], Steve Gauthier[2], Brian J. Day[1,2]*

1. Department of Pharmaceutical Sciences, University of Colorado Denver,, Aurora, CO 80045 USA.

2. Department of Medicine, National Jewish Health, Denver, CO 80206 USA.

Thiocyanate (SCN^-) is an endogenous small molecule derived from cyanide and found in μM concentrations in the extracellular fluids of the body. SCN^- in the blood, saliva, and epithelial lining fluid (ELF) can be utilized by multiple peroxidases (lactoperoxidase, LPO; myeloperoxidase, MPO; etc.) to scavenge hydrogen peroxide (H_2O_2) and react directly with hypochlorous acid (HOCl) resulting in the formation of hypothiocyanous acid (HOSCN). HOSCN is regarded as an antimicrobial defense factor and a less potent oxidant than HOCl, but has come under new scrutiny after reports of toxicity in animal cell lines. In this study, HOSCN is examined in multiple cell lines using both enzyme-driven and direct exposure systems and in comparison to HOCl. HOSCN demonstrated toxicity comparable to that seen with HOCl in the J774 murine alveolar macrophage cell line. Other cell lines were more resistant to HOSCN toxicity compared to HOCl. Intracellular glutathione (GSH) is a selective target of HOSCN but basal levels of GSH do not explain the sensitivity in J774. However, increasing intracellular GSH is protective against HOSCN toxicity in J774. While HOSCN can be toxic at high concentrations, steady state and low acute levels likely to occur in vivo appear to be less toxic to most cell types as compared with HOCl and may be a novel cell redox signaling agent.

Joshua Chandler, 417 880 5917, Joshua.chandler@ucdenver.edu

POSTER # 7

GRAPE SEED EXTRACT INDUCES APOPTOSIS IN HUMAN COLORECTAL CARCINOMA THROUGH MODULATION OF DEATH RECEPTORS AND ANTI-APOPTOTIC MOLECULES

Molly Derry, Rajesh Agarwal and Chapla Agarwal
Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver, Aurora, CO, USA

Colorectal Cancer (CRC) is the second leading cause of cancer related deaths in the US, with most (~75%) diagnosed cases resulting from sporadic CRC development. Diets rich in fruits and vegetables are shown to decrease CRC incidence by 40%, in part due to antioxidant and free radical properties of the polyphenolics present therein; grape seed extract (GSE) is one of the richest sources of these antioxidants. GSE has been shown to be non-toxic in clinical trials and exhibits numerous beneficial effects including anti-cancer efficacy. Oncogenic mutations that suppress apoptosis can lead to tumor initiation, progression, and metastasis; a major cause of conventional treatment resistance is due to apoptotic pathway defects. Accordingly, the strategies that target the mechanisms of apoptosis resistance in cancer cells provide the potential opportunities for cancer management. Herein we investigated the apoptotic effects of GSE and defined associated mechanisms in three human CRC cell lines, namely SW480, SW620 and HCT116, representing II-IV stages of CRC. Normal colon epithelial cells namely NCM460 was also included to establish GSE specificity, if any, towards CRC cells. A comparison of GSE activity in three CRC cell lines showed that almost three times higher GSE levels are needed for comparable cell death effect in SW480 versus SW620/HCT116 cells; cell treatments were accordingly adjusted for remainder studies. GSE treatment of SW480 cells at 25-100μg/mL resulted in a maximum of 55% apoptotic death at 48h; however, even 10-30μg/mL GSE treatments produced 53-57% apoptotic cell death in SW620 and HCT116 cell lines. At the molecular level, GSE induced DR4/5 up regulation, caspase-9, -3, -8, and PARP cleavage in all three cell lines indicating the involvement of both extrinsic and intrinsic apoptotic pathways in its efficacy. Furthermore, GSE treatment caused down regulation of anti-apoptotic proteins Bcl-2 and XIAP in SW480 cells only, but increased the levels of pro-apoptotic proteins such as Mcl-1s in all three cell lines. Importantly, GSE also increased the levels of p53 and its associated regulators namely p21, PUMA and Bak, suggesting their possible role in GSE-caused apoptotic death in HCT116 cell line. GSE treatment (25-100μg/mL) of NCM460 cells did not result in significant death compared to what was observed in three different human CRC cell lines, clearly showing its activity selectively in cancer cells. In conclusion, our findings further support the notion that GSE could be an effective chemopreventive and chemotherapeutic agent for colorectal cancer.
Molly Derry, Molly.derry@ucdenver.edu,303-724-4059

POSTER # 8

PROLONGED iAs EXPOSURE LEADS TO ABERRANT INSULIN SIGNALING IN L6 MYOCYTES

Ingrid L. Druwe, James J. Sollome, Richard R. Vaillancourt.
The Univeristy of Arizona, College of Pharmacy, Department of Pharmacology & Toxicology

Diabetes mellitus is a metabolic syndrome characterized by inappropriate production of insulin or the inability of cells to respond to insulin. It is estimated that by the year 2050 1 in 3 U.S. adults will have diabetes mellitus. Insulin is the principal hormone involved in lowering blood glucose and functions by suppressing liver gluconeogenesis and glycogenolysis in the liver and by stimulating the uptake of glucose into skeletal muscle and adipocytes. Recent epidemiological studies both in the USA and abroad have linked chronic ingestion of low levels of inorganic arsenic (iAs), an environmental toxicant, to the onset of diabetes mellitus. Although these observations have been met with some skepticism, there are few mechanistic studies have tried to elucidate the mechanisms by which iAs perturbs insulin signaling. Here we show that L6 myocytes, an insulin responsive cell line, exposed to low to moderate levels of iAs (0.25 to 2 μM) for 4 days show decreased insulin stimulated glucose uptake as well as increased phosphorylation of IRS-1 at serine 307, thereby impairing insulin stimulated uptake. These data support the epidemiological evidence that chronic exposure to low physiologically relevant levels of arsenite can contribute to insulin resistance and type 2 diabetes states. And while the etiology of type 2 diabetes has yet to be elucidated these data show that in addition to pharmacological treatment and lifestyle modifications, environmental exposures should also be considered when evaluating the etiology of type 2 diabetes.

Contact info: Ingrid Druwe 520-626-5671; druwe@pharmacy.arizona.edu

POSTER # 9

CYTOCHROME P450-MEDIATED DEMETHOXYLATION OF TRIAMCINOLONE ACETONIDE TO A CARBOXYLIC ACID; METABOLITE SYNTHESIS, PURIFICATION AND IDENTIFICATION.

Trevor Fidler, Chad Moore, Christopher Reilly and Garold Yost
Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

Triamcinolone Acetonide (TA) is a glucocorticoid commonly used in the treatment of pedantic asthma, but its efficacy is variable (i.e., steroid insensitivity) and there exists potential for systemic toxicity (i.e., adrenal suppression). It is hypothesized that differential metabolism and/or clearance of TA may underlie both phenomenon. The purpose of this study was to elucidate the metabolic fate/metabolite profile for cytochrome P450 3A4/5-dependent metabolism of TA in order to gain insight into mechanisms potentially effecting drug efficacy, particularly in respiratory tissues, versus systemic toxicity. In humans, the major metabolites of TA following an 800µg administration are 6β-hyroxy-TA, 21-carboxy-TA, and 6β-hydroxy-21-carboxy-TA, together representing ~90% of the excreted dose. Here we report the identification and characterization of a new CYP3A4/5-generated metabolite, 20-carboxy-TA, by comparison of the enzymatic product to a synthetic standard using mass spectrometry and proton-nuclear magnetic resonance spectroscopy. Currently, the pharmacological and toxicological properties of this metabolite are unknown, but we postulate that further characterization of the metabolism of TA using this and other metabolites as evaluation criteria may provide a greater understanding of processes that control TA disposition in human organs, thus providing new data to facilitate the development of improved therapies for asthmatics who may not be responsive to TA treatment.
Trevor Fidler, (575) 993-8427, Trevor.Fidler@utah.edu

POSTER # 10

QUANTUM MECHANICAL ANALYSIS OF 4-HNE-CYSTEINE ADDUCT STABILITY CHARACTERIZED BY CID / ETD TANDEM MASS SPECTROMETRY

Kristofer S. Fritz[1], Katherine A Kellersberger[2], Jose D. Gomez[1] and Dennis R. Petersen[1]

[1]Department of Pharmaceutical Sciences, Graduate Program in Toxicology, School of Pharmacy, University of Colorado Denver, Aurora, CO. [2]Bruker Daltonics Inc., Billerica, MA.

Sustained oxidative stress and resulting lipid peroxidation proves deleterious in numerous cellular pathways. A major lipoxidation product, 4-hydroxynonenal (4-HNE), is known to alter proteomic and genomic processes, contributing to numerous pathologies such as neurodegenerative diseases, alcoholic liver disease and cancer. Understanding physio-chemical mechanisms of 4-HNE reactivity may lead to new discoveries in biomarkers and therapeutics. Comparative collision-induced dissociation (CID) and electron transfer dissociation (ETD) tandem mass spectrometry was utilized to examine 4-HNE Michael adduct stability on Cys280 of human SIRT3. 4-HNE-Cys adducts are generally stable modifications, however, in this case, peptide fragmentation under CID conditions resulted in the neutral loss of 4-HNE, while ETD generated an abundance of peptide fragment ions without cleaving this labile adduct. CID adduct stability was achieved through reductive stabilization using sodium borohydride. Quantum mechanical parameters were calculated to examine atomic and energetic factors involved in the observed MS/MS fragmentations. The calculated lowest unoccupied molecular orbital (LUMO) was noticeably different among non-reduced and reduced 4-HNE adducted peptides. Based on these calculations the modeling software Spartan was utilized to visualize LUMO surface density, which demonstrates a sizeable shift in location and orientation around the 4-HNE-Cys280 moiety and provides significant insight into the characteristic CID fragmentation cleavage for the non-reduced 4-HNE adduct. While numerous known and unknown factors are likely involved in MS/MS fragmentation mechanisms, our findings suggest a potential role for electrostatic charge, E LUMO and LUMO surface density in the specific fragmentation characteristics of this 4-HNE-Cys Michael adduct. It is likely that numerous factors contribute to the stability of this 4-HNE adduct under MS/MS conditions and the observed neutral loss under CID fragmentation. Here, the calculated alterations in electrostatic charge and LUMO likely play a defining role in impacting the observed neutral loss of this 4-HNE-Cys adduct.

Kristofer Fritz, 303-724-3398, Kristofer.fritz@ucdenver.edu

POSTER # 11

COMPARATIVE TOXICOLOGICAL ANALYSIS OF QUANTUM DOTS ON HUMAN SKIN TISSUE

Jun Gao, Jennifer Hollingsworth and Rashi Iyer
Bioscience Division, Los Alamos National Laboratory, Los Alamos, New Mexico

Semiconductor quantum dots (QD) are of growing technological relevance with applications in photoelectronics, electronic integrated circuits, radiation tolerant solar cells, and biological labeling. We used an in vitro reconstructed human skin tissue equivalent, typically referred to as 3-D tissue culture or organotypic raft cultures, closely representing the complexity and structural integrity of human skin tissue system. In this study we performed a comparative analysis to determine size and composition effects of QDs on tissue responses. The QDs used were hydrophilic, negatively charged QDs with a Cadmium/Selenide (CdSe), Cadmium Telluride (CdTe) and Indium Phosphide core coated with mecaptoundecanoic acid (MUA). The sizes of these nanoparticles are 3nm, 5 nm small dots and 16 ± 5 nm large dots. In this study, QDs were topically applied to skin tissue at concentrations of 0.5µg/ml ~ 20 µg/ml to assess penetration, cellular viability, cytotoxicity and inflammatory responses. Our results implicate that the size and composition properties of QDs impact the ability of these nanomaterials to penetrate skin tissue and influence cytotoxic and inflammatory responses.

***Keywords* — Quantum dots, cell death, toxicology, skin tissue**

Jun Gao , 505-667-0409; jgao@lanl.gov, rashi@lanl.gov.

POSTER # 12

GENETICALLY DETERMINED INDIGENOUS AMERICAN ANCESTRY ASSOCIATES WITH ARSENIC METHYLATION EFFICIENCY IN AN ADMIXED POPULATION FROM MEXICO.

Gomez-Rubio P., Klimentidis Y. C., Cantu-Soto E., Meza-Montenegro M. M., Billheimer D., Lu Z., Chen Z., Klimecki W. T.

Great effort has been put into determining factors affecting human variation in arsenic metabolism, as many studies have shown that lower human arsenic methylation efficiency is associated with higher incidence of several arsenic-induced diseases. Studies have suggested that indigenous Americans (AME) methylate arsenic more efficiently, excreting significantly less % urinary monomethylarsonic acid (%uMMA); however data supporting this idea has been ambiguous. In this study we aimed to characterize the association between genetically estimated individual AME ancestry proportions and arsenic methylation efficiency in an admixed population (composed of two or more isolated ancestral populations) of 746 individuals environmentally exposed to arsenic in northwest Mexico. Arsenic exposure, measured as total urinary arsenic (uTAs) was 170.4 μg/L on average, and %AME mean and range in the population were 72.4 and 23-100, respectively. Adjusted (gender, age, AS3MT 7388/M287T haplotypes, body mass index (BMI), and uTAs) multiple linear regression model showed a statistically significant association relating higher individual AME proportion with lower %uMMA ($p < 0.01$). Univariate analysis showed a significant association between BMI and sex with %uMMA, while multivariate analysis revealed a significant interaction between these two variables indicating a negative association between BMI and %uMMA, significant only in females ($p < 0.01$). Additionally, age and AS3MT haplotypes carrying 7388 and M287T variants were also significantly associated with arsenic methylation efficiency ($p < 0.01$). This study shows, for the first time, the importance of genetically estimated ancestry in human arsenic metabolism. These data highlight the need to understand the complexity of individual variation in human arsenic metabolism in future epidemiologic studies, particularly those performed in admixed populations. (Funded by ES006694 and ES04940).

Information: Paulina Gomez-Rubio, 520 626 2598, gomezp@email.arizona.edu

POSTER # 13

GENETIC AND GENOMIC STUDIES INTO THE ROLE OF ATP1A2 IN BEHAVIORAL RESPONSES TO ALCOHOL

Stephanie M. Gritz, Colin Larson, Tzu L. Phang, Kirsten L. Floyd, and Richard A. Radcliffe - University of Colorado Denver

Alcohol abuse is a significant public health issue and understanding genetic factors related to the effects of alcohol in the brain may provide new avenues of treatment. We have been investigating the Na^+/K^+ exchanger Atpase1a2 (*Atp1a2*) for its potential role in responses to alcohol using the Inbred Short Sleep (ISS) and the Inbred Long Sleep (ILS) mouse strains, which have been selectively bred for differential alcohol sensitivity. *Atp1a2* is an integral membrane protein responsible for establishing and maintaining the electrochemical gradients of sodium and potassium ions across plasma membranes and is located in the brain, skeletal muscle, heart, and kidney. Microarray data have shown the ISS had 3.5 fold greater *Atp1a2* brain mRNA than the ILS. Alcohol treatment caused an increases of *Atp1a2* brain mRNA in both the ISS and the ILS. NextGen RNA sequencing (RNA-seq) in naive animals has shown 104 SNPs located throughout the gene, a truncated 3' UTR in the ILS, and in contrast to the microarray data, equal mRNA expression. The effects of alcohol on *Atp1a2* activity and protein expression are unknown. We sought to determine if the truncated 3' UTR and multiple SNPs throughout the gene had an effect on *Atp1a2* expression. Recombinant Inbred (RI) strains derived from ISS and ILS (LXS) were used in the experiment. Based on SNPs in the RI strains, we determined whether they exhibited the ISS or ILS genotype. We measured protein expression in naive male mice. Brains were harvested, homogenized, and hydrophobic proteins were extracted. *Atp1a2* protein expression was analyzed using Western Blotting. Preliminary data indicate the ILS-like genotype have an increased amount of protein compared to ISS-like genotype.

Stephanie Gritz, 303-724-3364, Stephanie.Gritz@UCDenver.edu

POSTER # 14

MOLECULAR MECHANISM OF ALTERED EZETIMIBE DISPOSITION IN NON-ALCOHOLIC STEATOHEPATITIS

Rhiannon N. Hardwick, Craig D. Fisher, Stephanie M. Street, Mark J. Canet, Nathan J. Cherrington
Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ

Ezetimibe (EZE) lowers serum lipid levels by blocking Neimann-Pick C1-Like 1-mediated cholesterol uptake in the intestine. Disposition its pharmacologically active glucuronide metabolite (EZE-GLUC) to the intestine is dependent on biliary efflux from the liver. The purpose of the current study was to determine the molecular mechanism by which disposition of clinically relevant drugs may be altered in NASH. Rats fed a methionine-choline deficient (MCD) diet for 8 weeks were administered 10 mg/kg EZE either by intravenous bolus or oral gavage. Plasma and bile samples were collected over 2 hours followed by terminal urine, liver and intestinal tissue collection in which Total EZE and EZE-GLUC concentrations were determined by LC-MS/MS. Hepatic expression of the sinusoidal Abcc3 transporter was induced in MCD animals which correlated with increased plasma concentrations of EZE-GLUC in MCD animals, regardless of dosing method. Hepatic expression of the biliary Abcc2 and Abcb1 transporters were also increased in MCD; and, the biliary efflux of EZE-GLUC was slightly diminished in MCD animals despite no alterations in the biliary concentration of total bile acids. Interestingly, the cellular localization of Abcc2 and Abcb1 appeared to be internalized away from the canalicular membrane in MCD livers, providing a mechanism for the shift to plasma drug efflux. Importantly, induction of ABCC2, 3 and ABCB1 protein was also found in human NASH livers. Similarly, ABCC2 also appeared to be internalized in human NASH. The combination of induced expression and altered localization of key efflux drug transporters in rodent and human NASH samples shifts the disposition profile of EZE toward plasma retention and greater exposure. This increased plasma retention of drugs in NASH could put these patients at greater risk for adverse drug reactions.
Rhiannon Hardwick, (520) 626-1531, hardwick@pharmacy.arizona.edu

POSTER # 15

EFFECTS OF CATECHOL-O-METHYLTRANSFERASE INHIBITION ON 3,4-METHYLENEDIOXYMETHAMPHETAMINE-INDUCED NEUROTOXICITY

Herndon JM, Cholanians AB, Lizarraga LE, Lau SS, and Monks TJ
Dept of Pharmacology and Toxicology, College of Pharmacy, University of Arizona Health Sciences Center, Tucson, Arizona 85721, USA.

Metabolism of 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) plays a major role in MDMA-induced serotonergic neurotoxicity. Thus, when injected centrally into the CNS, MDMA fails to recapitulate the neurotoxicity observed following peripheral administration. Cytochrome P450-mediated demethylenation of MDMA results in the generation of a catechol, N-methyl-α-methyldopamine (N-Me-α-MeDA), further metabolism of which is implicated in the generation of the ultimate neurotoxic species. We have therefore investigated the role of the phase II enzyme, catechol-O-methyltransferase (COMT) in MDMA-induced neurotoxicity. COMT catalyzes the O-methylation of N-Me-α-MeDA to 4-hydroxy-3-methoxy-methamphetamine, a reaction that competes with the oxdation of N-Me-α-MeDA to the corresponding reactive ortho-quinone and the subsequent formation of neurotoxic thioether metabolites. We utilized both a pharmacological and genetic model of COMT inhibition/ deletion to determine the effects of COMT on MDMA-induced neurotoxicity. Rats pretreated with the COMT inhibitor Ro 41-0960 displayed a 30% decrease in striatal serotonin content when compared to rats receiving MDMA alone (20mg/kg, sc). Mice carrying a single functional COMT allele (COMT +/-) dosed with MDMA (30mg/kg x3 at 3 hour intervals) displayed a 40% decrease in striatal dopamine compared to heterozygous mice receiving saline, whereas this decrease was only ~20% in WT (COMT+/+) mice. Serotonin levels in both the cortex and striatum were essentially unchanged between MDMA and saline treated mice. The findings indicate that COMT levels are a determinant of MDMA-induced neurotoxicity, an important finding given that COMT expression in the human population is polymorphic
Joe Herndon, herndon@pharmacy.arizona.edu, 520-626-9883

POSTER # 16

MECHANISMS OF SULFUR MUSTARD ANALOG 2-CHLOROETHYL ETHYL SULFIDE-INDUCED DNA DAMAGE IN SKIN EPIDERMAL CELLS AND FIBROBLASTS

Swetha Inturi[1], Neera Tewari-Singh[1], Mallikarjuna Gu[1], Sangeeta Shrotriya[1], Joe Gomez[1], Chapla Agarwal[1], Carl W. White[2], Rajesh Agarwal[1]
1 Department of Pharmaceutical Sciences, University of Colorado Denver
2 Department of Pediatrics, National Jewish Health

Employing mouse skin epidermal JB6 cells and dermal fibroblasts, here we examined the mechanisms of DNA damage by 2-chloroethyl ethyl sulfide (CEES), a monofunctional analog of SM. CEES exposure caused H2A.X and p53 phosphorylation as well as p53 accumulation in both cell types starting at 1 h that sustained till 24 h, indicating a DNA-damage effect of CEES, which was also confirmed and quantified by alkaline comet assay. CEES exposure also induced an oxidative stress and oxidative DNA damage in both cell types, measured by an increase in mitochondrial and cellular reactive oxygen species, and 8-hydroxydeoxyguanosine levels, respectively. In the present study to distinguish between oxidative and direct DNA damage, we have used 1 h pretreatment with glutathione (GSH) or antioxidant trolox where both the agents showed a decrease in CEES-induced oxidative stress and oxidative DNA damage. However, only GSH pretreatment caused a decrease in CEES-induced total DNA damage measured by comet assay, H2A.X and p53 phosphorylation, and total p53 levels. This was possibly due to the formation of GSH-CEES conjugate detected by LC-MS analysis. Together, our results show that although CEES causes both direct and oxidative DNA damages, oxidative stress is not a prominent contributor to the CEES-induced DNA damage, suggesting that to rescue SM-caused skin injuries, pleotropic agents (or cocktails) are needed which could target multiple pathways of mustard skin toxicities.
Swetha Inturi, 303-724-4059, Swetha.inturi@ucdenver.edu

POSTER # 17

HUMAN ALDH1B1: ADDITIONAL SUBSTRATES AND MOLECULAR MODELING OF POLYMORPHISMS

Brian Jackson, Philip Reigan and Vasilis Vasiliou

Background: A partial substrate profile for ALDH1B1 has been determined previously and includes acetaldehyde and other short-chain aldehydes, but not lipid peroxidation products. However, some evidence suggests that additional substrates for ALDH1B1 may exist, including nitroglycerin and retinaldehyde. Individuals deficient in ALDH2, a key enzyme in nitroglycerin metabolism, still retain as much as 30% efficacy from nitroglycerin. In addition, ALDH1B1 is implicated in stem cell biology and may play a role in development, raising the possibility that it may mediate retinaldehyde signaling. Three common ALDH1B1 polymorphisms have been reported in human population. These ALDH1B1 variants may affect its enzymatic activity. Epidemiological studies have shown ALDH1B1*2 individuals to have ethanol avoidance, increased systolic blood pressure, and ethanol hypersensitivity reactions, such as itchy runny nose, sneezing, shortness of breath, rash, itching or swelling. ALDH1B1*3 had no associations with any of the parameters tested in epidemiological studies. ALDH1B1*5 has not been studied.
Aims: To determine if nitroglycerin can be metabolized by ALDH1B1 at saturating concentrations. To determine the kinetic parameters of metabolism of all-trans retinaldehyde by ALDH1B1. To use molecular modeling to 1) provide a physicochemical basis for observed epidemiological differences and 2) predict differences in metabolism that might arise from polymorphic variants.
Results: ALDH1B1 metabolizes and appears to be inhibited by nitroglycerin. ALDH1B1 has favorable kinetics for all-trans retinaldehyde in vitro. ALDH1B1 metabolism of key substrates including acetaldehyde, nitroglycerin, and all-trans retinaldehyde may not occur in some variants due to poor binding profiles.
Conclusions: Here we expand knowledge of this key enzyme by characterizing the metabolism of two additional substrates. Molecular modeling of acetaldehyde binding to ALDH1B1 variants is consistent with epidemiological studies. Molecular modeling of other substrates predicts catalytic deficiency that may affect the physiological role of ALDH1B1.
Brian Jackson, 303 724 3521, brian.jackson@ucdenver.edu

POSTER # 18

THE CONTRIBUTION OF CYP3A P450 ENZYMES TO THE DIFFERENTIAL METABOLISM OF BUDESONIDE

Jana James and Garold Yost, University of Utah, Salt Lake City, UT

According to the current asthma trends outlined in the National Center for Health Statistics 2011 report, as of 2009 24.6 million (8.2%) of Americans battle with this inflammatory disease. Inhaled glucocorticoid steroids, such as budesonide, are regularly prescribed for the treatment of asthma due to their potent anti-inflammatory actions that reduce inflammation and hyper-reactivity of the airways. Following oral administration and adsorption, budesonide is subject to a high first pass metabolism (80-90%) namely by the CYP3A P450 enzyme family. During metabolism, we believe that catalytic mechanisms of facilitated electron transport that generate dehydrogenation by certain P450 enzymes results in xenobiotic-meditated injury and altered drug metabolism in humans. However, the mechanism of cytochrome P450-mediated dehydrogenation of budesonide in processes that generate toxic electrophilic intermediates is unknown. The aim of this present work was to develop a LC-MS-MS analytical method to identify major budesonide metabolites produced by microsomal and supersomal (CYP3A4, 3A5, 3A7) enzyme incubations. Simultaneously, molecular mechanic docking simulations were used to integrate in silico and biochemical methods as a way to establish the mechanisms of dehydrogenation through enzyme/ substrate interactions. Adduct formation of the dehydrogenated metabolite (Δ6-BUD) through glutathione (GSH) sequestration in supersomal incubations using LC-MS-MS was also investigated. We have evidence to show that a thioether adduct is formed and this could possibly indicate a toxic protein adduct formation, but further studies would need to be conducted to determine structure and mechanisms of toxicity.

Jana James, Phone: 801-581-8089, Email: jana.james@utah.edu

POSTER # 19

CHARACTERIZATION OF AN ASTROCYTE SPECIFIC IKK-BETA KNOCKOUT MOUSE.

Kelly Kirkley1 and Ronald Tjalkens1.

1. Center of Environmental Medicine, Colorado State University, Fort Collins, CO.

The release of pro-inflammatory mediators by activated astrocytes and microglia have been documented in neurodegenerative diseases such as Parkinson's disease and manganese-induced neurotoxicity; however, the relative impact of these activated glia and the pathways involved is still controversial. In this study, we bred an hGFAP-Cre mouse line with a floxedIkk-beta mouse line to create a conditional Ikk-beta knockout mouse (/IKK-/-) in which the Ikk-beta gene is specifically deleted in astrocytes in the CNS. In vivocomparison of Ikk-beta staining in adult knockout mice to wild type (IKKF/F) littermates revealed maintenance of the Ikk-beta in neuronal populations of the basal ganglia, but staining was unsuccessful in glial populations. In vitro staining revealed deletion of Ikk-beta in primary cultured astrocytes, but not in primary cultured microglia. Semi-quantitative analysis revealed at least a 50% reduction in Ikk-beta activity in primary cultured astrocytes as compared to wild-type controls. Current data suggests the mouse has been successful in specifically deleting Ikk-beta within astrocytes of the CNS and should be a useful tool to determine the role of astrocyte Ikk-beta in neurodegeneration.

Kelly Sullivan Kirkley, 970-491-2771, Kelly.anne.sullivan@colostate.edu

POSTER # 20

TRANSPORTER-MEDIATED MECHANISM OF NUCLEOSIDE PENETRATION OF THE BLOOD-TESTIS BARRIER

David M. Klein*, Kristen K. Evans†, Rhiannon N. Hardwick*, William H. Dantzler†, Stephen H. Wright†, Nathan J. Cherrington*
University of Arizona, Departments of *Pharmacology & Toxicology and †Physiology

Drugs used to treat HIV are effective at reducing viral load in circulating blood but are unable to eliminate the virus due to biological sanctuary sites such as the brain or testis. The blood-testis barrier (BTB) prevents the entry of many therapeutics into the lumen of the seminiferous tubules (STs), shielding semen from chemical exposure. The BTB is formed by tight junctions between Sertoli cells, the epithelial cells of the STs. One drug class of HIV therapeutics, nucleoside analogs (NSAs), can penetrate the BTB and be detected in the semen. The purpose of this study is to determine the mechanism by which NSAs are transported across the BTB. Transport studies in isolated rodent STs using H3uridine (Kt 89.72uM) as a model nucleoside substrate indicate that Sertoli cells take up nucleosides almost exclusively via equilibrative nucleoside transporter 1 (ENT1), which transports nucleosides across membranes down their concentration gradient (IC50 for NBMPR, an ENT1 inhibitor, is 12.9 nM). These data correspond with immunohistochemical staining of rat testes which show ENT1 on the basolateral membrane, whereas ENT2 is on the apical membrane of Sertoli cells. This localization suggests that ENT1 acts as an uptake transporter and ENT2 may facilitate the efflux of NSAs into the lumen of STs. Uptake of didanoisine, an NSA commonly used to treat HIV, in HEK cells can be blocked by ENT1 inhibition,. These data indicate a novel ENT-dominant mechanism for the transepithelial transport of nucleoside analogs across the BTB.
David Klein, 678-446-2413, Klein@pharmacy.arizona.edu

POSTER # 21

MOLECULAR MODELING STUDIES ON HUMAN ALDH1A1-DRUG INTERACTIONS

Koppaka V, Reigan P and V. Vasiliou
Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045

Aldehyde dehydrogenase 1A1 (ALDH1A1) belongs to the aldehyde dehydrogenase superfamily of enzymes which irreversibly catalyze the NAD(P)+-dependent oxidation of both endogenous and exogenous aldehydes to their corresponding carboxylic acids. High ALDH activity has been associated with increased cyclophosphamide (anti-cancer) drug resistance through drug metabolism, tumorigenic potential and relapse in patients following chemotherapy. Non-enzymatic functions of ALDH1A1 were recently identified to include the ability to bind endobiotics (androgen, thyroid hormone and cholesterol) and xenobiotics such as quinoline drugs. Certain acute myeloid leukemia drugs including Daunorubicin and Flavopiridol were also shown to bind ALDH1A1. In this preliminary study, we examined ALDH1A1 – drug interaction via molecular modeling using four candidate drugs, Flavopiridol, Ara-C, Clofarabine and Fludarabine and homology model of human ALDH1A1protein. Docking poses indicate that Flavopiridol binds with a higher affinity relative to the other drugs. The binding site is located close to the coiled-coil motif with participating residues from both the NAD+ binding and oligomerization domains, which may subsequently inhibit activity and dimerization of the monomers. In conclusion, ALDH1A1 could mediate cancer relapse through drug interaction.

POSTER # 22

POST TRANSLATIONAL PROTEIN MODIFICATIONS AND CANCER: INVESTIGATING THE IMPACT OF PROTEIN ADDUCTION BY REACTIVE ELECTROPHILES IN BENZENE-MEDIATED HEMATOTOXICITY.

C Kuhlman[1], DR Petersen[2], TJ Monks[1], SS Lau[1].
[1]Pharm/Toxicol, Pharmacy, Southwest Environmental Health Sciences Center, U. of Arizona, Tucson, AZ, [2]Pharm. Sci., U. of Colorado, Denver, CO,

It is well supported that the redox-active and electrophillic metabolites of benzene contribute to its myelotoxic effects. When administered to rats or mice, a combination of two benzene metabolites, hydroquinone (HQ), and phenol (PHE), reproduces benzene myelotoxicity. HQ readily oxidizes to 1,4-benzoquinone (1,4-BQ) and in the presence of glutathione (GSH) gives rise to multi-GSH substituted conjugates, several of which are present in the bone marrow of rats or mice following PHE/HQ (1.1/0.9 mmol/kg, ip) administration. All of the HQ-GSH conjugates retain the ability to arylate proteins and to redox cycle. Indeed, the multi-GSH substituted conjugates of HQ are far more efficient generators of superoxide anion than the HQ/1,4-BQ redox couple. However, the precise molecular mechanism by which benzene and HQ-GSH conjugates induce hematotoxicity remains to be determined. Following co-administration of PHE/HQ to rats, blood lymphocyte counts were significantly decreased (58-89%). Furthermore, malondialdehyde (MDA) levels measured in bone marrow lysate showed a 15 fold increase in treated rats compared to control. Using anti-(GS/NACys)HQ and anti-4-hydroxy-2-nonenal (4HNE) specific antibodies on 1D/2D westerns, followed by MS/MS peptide sequencing analysis of immunopositive proteins from bone marrow, we revealed the presence of 35 (GS/NACys)HQ- and 4HNE-adducted proteins. Moreover, HQ-GSH (E, K, R) and 4HNE (C, H, K) adduction sites were identified on 10 proteins. HQ-GSH adducted hemoglobin-a,b (Hb), 14,3-3 protein zeta/delta, ribose 5-phosphate isomerase A (RPI), b-, a--enolase, annexin A-1, tropomyosin--a4 and nonO/p54nrb while 4HNE modified Hb, 14,3-3 protein zeta/delta, RPI, carbonic anhydrase 1, and bisphosphoglycerate mutase. Analysis of the structural and functional significance of these protein modifications may provide new insight into the progression of benzene-induced leukemia. Of interest for further analysis is 14-3-3 protein, which has been reported to act as a histone deacetylase inhibitor. Silencing/down regulation of 14-3-3 protein has been observed in cancers of the lung, bladder, and liver. Adduction of 14-3-3 following benzene exposure may contribute to chromatin remodeling abnormalities and genomic instability. The functional alteration of 14-3-3 and the possible role it plays in benzene-induced leukemia is currently under investigation. (P30ES006694, RO1GM70890, Sci. Found. of AZ, T32ES007091)
Christopher Kuhlman, 805-890-9143, kuhlman@pharmacy.arizona.edu

POSTER # 23

CHANGES IN GENE EXPRESSION IN PCB-EXPOSED AND REFERENCE WILD RAINBOW TROUT

James LaVelle, Ph.D., CDM, Tucson, AZ; Madison Powell, Ph.D., Aquaculture Research Institute, University of Idaho, Hagerman, ID; Tamzen Macbeth, Ph.D., CDM, Helena, MT; Tony Gendusa, Ph.D., CDM, Little Rock, AR

An ongoing problem in risk assessment is the ability to associate toxicity to ecological receptors with contaminants in sediments. Often, such assessment uses weight of evidence, which may include chemical concentrations in abiotic media and in tissues, laboratory or in situ bioassays, and analyses of community structure. Laboratory studies suggest that a fourth line of evidence might also prove useful. Some common contaminants induce reproducible patterns of gene expression in tissues, along with measurable toxicity, in exposed fish,. If such patterns could be recognized in wild populations, substantial information could be provided on what chemicals are present in biologically important concentrations. As a first step, a site in Montana was selected where a single group of contaminants, PCBs, was present in sediments. Rainbow trout (Oncorhynchus mykiss) were collected from both the most contaminated reach of the creek and a nearby reference stream in the same watershed. Samples of liver and other tissues were processed at the Aquaculture Research Institute of the University of Idaho. Gene expression was assessed using a microarray developed for rainbow trout at the University of Victoria. Relative gene expression across the array was examined for sets of both down-regulated and up-regulated genes in exposed male fish as compared to reference males. In liver tissue, a variety of possible targets for PCB effects was identified, which included genes associated with basal metabolism, hormone regulation, RNA-binding proteins, metallothionein and others. The results suggest that PCB exposure could cause an array of changes in gene expression that might ultimately be related to toxicity in the field. However, additional studies will be necessary to determine whether some of the differences in gene expression could be related to differences in habitat, water quality or other factors between the study and reference areas that were not obvious in the field. Microarray results from other tissues, such as gill, muscle, testes/ovaries and skin might provide additional insight, and these assays are being run. However, it will likely prove necessary to perform a more controlled study in the laboratory to clearly identify PCB-related changes in gene expression.

James LaVelle, 520-625-3151, lavellejm@cdm.com

POSTER # 24

INHIBITION OF THE SEROTONIN AND VESICULAR MONOAMINE TRANSPORTERS ATTENUATES METHYLENEDIOXYMETHAMPHETAMINE- INDUCED NEUROTOXICITY

Lizarraga LE, Herndon JM, Cholanians AB, Lau SS, and Monks TJ
Dept of Pharmacology and Toxicology, College of Pharmacy, University of Arizona Health Sciences Center, Tucson, Arizona 85721, USA.

3,4-Methylenedioxymethamphetamine (MDMA, Ecstacy) is a ring substituted amphetamine derivative with potent stimulant properties. MDMA long-term serotonergic neurotoxicity manifests as a prolonged depletion in serotonin (5-HT) and structural damage to 5-HT axons. The former effect is likely mediated via the ability of MDMA to inhibit 5-HT reuptake via the 5-HT transporter (SERT), since fluoxetine, a selective 5-HT reuptake inhibitor, protects against MDMA-induced ROS generation and neurotoxicity. We have now investigated the effect of SERT deficiency on MDMA-mediated neurotoxicity using a SERT-KO rat model. SERT-KO rats dosed with MDMA (20mg/kg, sc) exhibit a 20-50% increase in striatal and cortical 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations compared to SERT-KO receiving saline. In contrast, wild-type (WT) rats dosed with MDMA (20mg/kg, sc) exhibited an ~50% decrease in both 5-HT and 5-HIAA compared to the WT saline treated group. Thus, SERT-KO rats showed resistance against MDMA-mediated depletion of 5HT/5-HIAA concentrations. In contrast to the SERT, the vesicular monoamine transporter (VMAT2) is involved in the loading of monoamine neurotransmitters, in particular dopamine (DA) and 5-HT, into intra-neuronal storage vesicles. As such, VMAT2 is critical in maintaining neuronal health by preventing neurotransmitter oxidation within the cytosol. Indeed, low expression VMAT2 mice (VMAT2 LO) are more susceptible to methamphetamine-induced neurodegeneration than are WT mice. We therefore investigated the effects of the pharmacological inhibition of VMAT2, using Ro 4-1284, on MDMA-mediated neurotoxicity. Sprague-Dawley rats receiving the VMAT2 inhibitor pretreatment (10mg/kg, ip) displayed a 50% increase in 5-HT content in the cortex and striatum compared to rats that were dosed with MDMA (20mg/kg, sc) alone. Thus, pharmacologic inhibition of VMAT2 attenuates MDMA-mediated depletion of 5HT/5-HIAA concentrations. In summary, SERT and VMAT2 likely act in concert to protect neurons from the adverse effects of MDMA.
Contact: Lucina Lizarraga, lizarrag@pharmacy.arizona.edu, 520-626-9883

POSTER # 25

ANTI-TUMORIGENIC EFFECTS OF SILIBININ IN COMBINATION WITH HISTONE DEACETYLASE INHIBITORS (TSA AND SAHA): INVOLVEMENT OF G2/M ARREST AND APOPTOTIC CELL DEATH

Samiha Mateen[1], Mallikarjuna Gu[1], Anil Jain[1], Chapla Agarwal[1, 2,] Daniel Chan[2] and Rajesh Agarwal[1, 2]
[1]Department of Pharmaceutical Sciences, School of Pharmacy, [2]University of Colorado Cancer Center, University of Colorado Denver Aurora, CO 80045, USA

Deregulated histone alterations, (particularly those involving HDAC [histone deacetylase] enzyme) are hallmark epigenetic characteristics of a transformed/malignant cell phenotype. Due to their several histone and non-histone substrates and multi-faceted regulatory role in biological events such as cellular proliferation, migration, angiogenesis as well as control of gene expression, targeting HDACs using HDAC inhibitors (HDACi) alone or in combination with other therapies has emerged as an attractive therapeutic option in a broad spectrum of diseases including Non-small cell lung cancer (NSCLC). NSCLC comprises of ~80% of newly diagnosed cases of lung cancer and current therapies including chemotherapy and HDACi are moderately efficacious in the treatment of this lethal disease. Our aim in the present study is to demonstrate the efficacy and related mechanisms of two pan-HDAC inhibitors (Trichostatin A (TSA) and SAHA) alone and in combination with Silibinin (a non-toxic chemopreventive and anti-cancerous phytochemical, isolated from the seeds of milk thistle) both in vitro and in vivo. H1299 cells concurrently treated for 24-72h with 75µM Silibinin (Sb) and HDACi (0.5µM TSA or 5 µM SAHA) showed consistent cell growth inhibition accompanied with significant increase in cell death as when compared to treatment with single agent alone. Further evaluation, at similar time points, using the same combination treatments showed a strong arrest of cells in G2/M phase of cell cycle and a linear increase in apoptotic cell population indicating the mechanism/s underlying the anti-proliferative effects. We examined the protein expression profiles of prosurvival molecules that have been shown to modulate sensitivity of cancer cells to apoptosis. At the molecular level, addition of silibinin to either TSA or SAHA resulted in a decrease of anti-apoptotic proteins including Bcl-xl, Bcl-2, Mcl-1, XIAP and survivin suggesting their mechanistic involvement in the apoptosis mediated by combined drug exposure. Concomitant cleavage of caspases (9 and 3) and PARP and a marked induction of p21 and altered phospho-cdc2 levels (48h) re-affirmed these observations, in H1299 cells. We also assessed the in vivo tumorigenic potential of these combinations in athymic nude mice (six mice per group) in terms of their xenograft growth for four weeks. An intraperitonial injection of both HDACi i.e. TSA (0.8mg/kg body weight) or SAHA (100 mg/kg body weight) and oral Silibinin (100 mg/kg body weight), was administered alone or in combination every day. At the end of the treatment regimen, as compared to the control (956 mm3 tumor volume/mouse), and single agent treatment groups i.e. Silibinin alone (408 mm3 tumor volume/mouse), TSA alone (495 mm3 tumor volume/mouse) and SAHA alone (377 mm3 tumor volume/mouse), a significant reduction in tumor volume was observed in the combination treatment groups i.e. TSA + Sb (486 mm3 tumor volume/mouse) and SAHA + Sb (208 mm3 tumor volume/mouse; $p<0.05$). Overall, both in vitro and in vivo results implicate the promising use of silibinin along with HDACi in the treatment of NSCLC. (Supported by CA113876).
Samiha Mateen, 303-724-4067, samiha.mateen@ucdenver.edu

POSTER # 26

ALDEHYDE DEHYDROGENASE 1B1 IN HUMAN COLON CANCER CELL LINES

Akiko Matsumoto, Ying Chen and Vasilis Vasiliou
Department of Pharmaceutical Sciences, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA

The aldehyde dehydrogenase (ALDH) superfamily contains nineteen proteins that catalyze the oxidation of endogenous and exogenous aldehydes to the corresponding carboxylic acid. Recent studies have revealed that ALDH activity is a highly specific marker for identifying stem cells within normal and tumorous tissues. Furthermore, the human ALDH1A1 isozyme is higly upregulated within many highly malignant cancer types.

Another ALDH isozyme, ALDH1B1, was recently identified and characterized biochemically, but its physiological roles remain elusive. We recently reported that ALDH1B1 is more highly expressed in human colon adenocarcinomas than ALDH1A1. In addition, ALDH1B1 was found to metabolize retinaldehyde to retinoic acid; an important nuclear ligand that signals cell differentiation. We hypothesize that the ALDH1B1-mediated synthesis of retinoic acid plays a significant influences colon cancer cell fate and disease progression.

The mRNA expression profiles for ALDH isozymes were determined by qRT-PCR in human colon cancer cells (BE, CACO-2, COLO320DM, HCT116, HT29 and SW480). Protein and ALDH activity levels were also assessed. ALDH1A1, 1A2, 1A3, 1B1 and 2 were found to be more highly expressed than ALDH3A1, 3A2 and 3B1. Moreover, ALDH1B1 and ALDH2 were highly expressed in every cell line and were more enriched within stem cell populations (CD44+ALDH+). Knockdown of ALDH1B1 with siRNA increased cell proliferation rates.

Results from these studies revealed that ALDH1B1 is highly expressed in human colon cancer cell lines, particularly within stem cell populations. Our data also indicates that ALDH1B1 has an important role in cancer cell expansion. Further studies are needed to better understand this role.

Akiko Matsumoto 303-724-3521 Akiko.Matsumoto@ucdenver.edu

POSTER # 27

VANADIUM COMPLEXES ANALYZED FOR NOVEL THERAPEUTIC POTENTIAL

Cameron McElroy[1], Remy Kachadourian[1], Debbie C. Crans[2], Phil Pharazyn[2] Brian J. Day[1]
[1]Department of Medicine, National Jewish Health, Denver, CO 80206, USA,
[2]Department of Chemistry, Colorado State University, Fort Collins, CO, 80523-1872, USA

Metalloporhyrin complexes can exhibit a wide variety of antioxidant effects in vitro and in vivo. The antioxidant effects are closely linked to the metal ion contained within the complex which gives the compounds ability to give and take electrons. As a follow-up to this research, 35 compounds were synthesized with vanadium for evaluation of possible antioxidant activity. The thiobarbituric acid reactive species test (TBARS) was used to screen the oxidative capabilities of compounds at concentration ranges between 0.1μM and 1000 μM. The lipid peroxidation reaction was initiated using an iron/ascorbate solution, and the experiments were carried out in rat brain tissue due to its high lipid content. Three of the vanadium compounds were found to possess moderate antioxidant properties. The strongest antioxidant detected in this study was VVO(hshed)(Cat), which suppressed 88% of the lipid oxidation. Surprisingly, many of the vanadium compounds tested displayed pro-oxidant effects in the TBARS assay, the strongest of which (VIIICl2(acac)(BP), showed a dramatic 6-fold increase in lipid oxidation over the control. Further evaluation of VVO(hshed)(Cat) is warranted for its potential use as an antioxidant to treat oxidative stress in cell culture and animal models of disease. The pro-oxidant compounds may find a place in causing oxidative damage to tumors as potential chemotherapeutic, while the weaker pro-oxidants may be useful for stimulating an adaptive antioxidant response as Nrf2 inducing agent.

POSTER # 28

An LC-MS/MS Method for Determination of Various Drugs of Abuse and Metabolites in Wastewater Effluent Samples

Ryley McPeters, Jennifer Berkbigler, Greg Dooley
Center for Envirnmental Medicine, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, 80523

A method was developed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification and confirmation of 8 drugs of abuse (cocaine, codeine, MDMA, methadone, methamphetamine, morphine, nicotine, and oxycodone) and various metabolites (acetylmorphine, cotinine, EDDP, amphetamine and benzoylecgonine) in municipal wastewaters. Drugs were extracted from wastewater samples with a pre-treatment consisting of solid phase extraction using Oasis MCX cartridges. Ultra-high-pressure liquid chromatography (UPLC) and positive electrospray ionization was used along with dynamic multiple reaction monitoring (MRM) tandem mass spectrometry for identification and quantitation. The extraction method was validated with matrix matched spiked samples at the limits of quantitation (0.5 ng/mL to 30 ng/mL) for each analyte with recoveries ranging from 71%-115%. Deuterated internal standards for each analyte were used to correct for errors that may have resulted from matrix effects, ion suppression, or sample preparation. The validated method was applied to municipal wastewater samples collected by grab sampling daily at a point source effluent into Fossil Creek, Fort Collins, CO. Eight of thirteen drugs being measured were found on a daily basis with the maximum being 2.15 ng/mL of morphine. Samples showed various spikes in drug concentration at 7 day intervals that corresponded with weekends.
Ryley McPeters, 970-491-5128, ryley.mcpeters@colostate.edu

POSTER # 29

Assessment of Nuclear Factor Kappa B (NF-κB) Signaling in the Hippocampus During Kanic Acid Exposure Using NF-κB reporter mice.

James A. Miller1, Russell A. Bialecki[2], Ronald B. Tjalkens[1]
[1]Center for Environmental Medicine, Colorado State University, Fort Collins, CO, USA
[2]AstraZeneca Safety Assessment, Wilmington, DE, USA

NF-κB, is a key transcriptional regulator of numerous genes including those involved in regulating cell death / survival pathways and cellular plasticity. Chemical induced kindling of seizure activity results from sustained alterations in neuronal excitability, indicative of neuronal plasticity resulting in hyper-excitability. In the present study, we employed a unique transgenic reporter mouse expressing NF-κB dependent GFP, in order to investigate the role of NF-κB signaling in rendering hippocampal neurons hyper-excitable. Mice were treated with kainic acid (KA, 2 x 10 mg/Kg, ip) over 48 hr and NF-κB activation was assessed 24 hours following treatment. Animals displayed mild behavioral changes consistent with the early stages of seizure induction including generalized immobility and facial clonus which progressed to include mild head nodding after the second dose of KA. Assessment of reporter expression showed that under basal conditions GFP is absent in the hippocampus except for a pronounced expression in the CA3 region pyramidal layer. After KA treatment, increased expression of GFP in the CA3 region and marked expression in the stratum moleculare, dentate gyrus molecular layer and in the dentate hilus were observed. These preliminary data show selective regional effects of KA on NF-κB activation which mimic the sensitivity of these regions to KA induced excitoxicity. This demonstrates the potential utility of this reporter model in investigating NF-κB activation in seizure induction and maintenance and may also prove useful in detecting chemically-induced effects on the hippocampus that may underly seizuregenic activity of pharmaceutical compounds.
James Miller, (970)491-2771, james.miller@colostate.edu

POSTER # 30

INCREASED SUSCEPTIBILITY OF CD1D -/- MICE TO APAP-INDUCED LIVER INJURY

Brittany V Murphy and Cynthia Ju.
Department of Pharmaceutical Sciences, University of Colorado Denver, Aurora, CO 80045, USA.

The idiosyncratic nature, severity and poor diagnosis of drug-induced liver injury (DILI) make these reactions a major safety concern during drug development, as well as the most common cause for the withdrawal of drugs from the pharmaceutical market. Evidence suggests that aside from drug-induced direct damage to hepatocytes, an inflammatory innate immune response is triggered that may contribute to the overall pathogenesis of liver injury. The specific role that natural killer T (NKT) cells play in acetaminophen (APAP)-induced liver injury remains the topic of much controversy. CD1d is a major histocompatibility complex (MHC) class I–related molecule that functions in glycolipid antigen presentation to NKT cells allowing for their activation and development.

We found that upon APAP-challenge, CD1d deficient mice (lack of NKT cells) are more susceptible to hepatic injury compared with WT mice. Higher levels of APAP-protein adducts were observed in liver homogenates and mitochondrial fractions prepared from APAP-treated CD1d -/- mice compared to WT mice, this appears to be due to the up-regulation of CYP2E1 protein expression and activities in CD1d -/- mice following starvation. In addition, starvation led to mitochondrial dysfunction and increased ROS in CD1d -/- mice. Furthermore, ER stress was observed in CD1d -/- mice, but not in WT mice, following starvation and various time points after APAP-treatment.

Collectively, our data demonstrate that compared with WT mice, CD1d-/- mice are more susceptible to mitochondrial oxidative stress and ER stress, thereby exacerbating liver injury upon APAP-challenge.

Brittany Murphy, 303-724-4025, Brittany.murphy@ucdenver.edu

POSTER # 31

KINETICS OF CYP P450 3A ENZYMES IN METABOLIZING BECLOMETHASONE DIPROPIONATE

Jessica K Roberts and Garold Yost, University of Utah, Salt Lake City, UT

Asthma is a disease that causes chronic inflammation of the airways, bronchoconstriction, increased mucus production and occasional airway obstruction. As of 2009, 24.6 million people were reported as being afflicted with asthma, with 7 million of those being children under the age of 18. The mainstay treatments for asthma are inhaled glucocorticoids. The efficacy of these drugs is due to their action on the glucocorticoid receptor in the lung which decreases the gene expression of inflammatory agents as well as mucus production. Although these treatments are effective in much of the population, about 30% of patients do not respond to treatment. It has been postulated that treatment is not effective due to glucocorticoid resistance or insensitivity, but there may be a different explanation based on the metabolism of the drug. Previous studies showed that these drugs are metabolized by 3A enzymes and esterases. Beclomethasone dipropionate (BDP), in particular, is administered as an ester pro-drug. Esterases in the lung cleave off an ester at the 17 position to make the drug biologically active. 3A enzymes also metabolize the drugs and facilitate the clearance of the BDP, and may also be responsible for the hydrolysis of the ester at position 17. It is possible that an equal balance of these enzymes working together contribute to the biological efficacy of BDP. The goal of this study was to determine the kinetics of the three different CYP P450 enzymes, namely 3A4, 3A5 and 3A7, in metabolizing beclomethasone dipropionate. Incubations with supersomes were performed for 20 minutes where aliquots were taken every five minutes. Aliquots were analyzed using LC/MS/MS to measure the formation of metabolites, as well as the disappearance of the parent compound. Esterase inhibitors were used in these experiments to evaluate the kinetics of just the p450 enzymes. From these experiments, it was found that 3A4 and 3A5 metabolized BDP at a similar rate where 3A7 did not metablolize BDP at all. These rates contribute to our understanding of the metabolism of these drugs in the lung, especially when looking at neonatal and pediatric populations, since these 3A enzymes are expressed at varying levels as compared to adults. This work aids in the understanding of the metabolism of BDP in the lung, and further studies in in vitro cell culture work is needed to corroborate these findings.

Jessica Roberts, 801-581-8089, jessica.roberts@utah.edu

POSTER # 32

GRAPE SEED EXTRACT INDUCES DNA DAMAGE CAUSING APOPTOSIS AND G2/M ARREST IN HEAD AND NECK SQUAMOUS CELL CARCINOMA: ROLE OF REACTIVE OXYGEN SPECIES AS POTENTIAL MEDITOR

Sangeeta Shrotriya, Gagan Deep, Mallikarjuna Gu, Anil Jain, Swetha Inturi, Chapla Agarwal, and Rajesh Agarwal
Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver, Aurora, CO, US
According to National Cancer Institute, head and neck squamous cell carcinoma (HNSCC) accounts for 6 percent of all malignancies in the USA with approximately 49, 260 new cases and 11, 480 deaths were expected in 2010 alone. In HNSCC, radiation and chemotherapy are the conventional treatment options available. Unfortunately, recurrence of disease after chemo/radiation therapy, toxicity associated with therapy and development of resistance against conventional treatments altogether decrease the 5-year survival rate in HNSCC patient by 50%. Therefore, use of non-toxic chemopreventive agents could be a promising approach to inhibit and/or delay the progression of various cancers including HNSCC. In the present study, we investigated the efficacy of grape seed extract (GSE) and associated mechanism in human HNSCC cells namely, Detroit 562 and FaDu using both in vitro and in vivo models. Cell culture studies revealed that GSE treatment (20 and 40 μg/mL) significantly inhibits cell growth, arrests the cells in G2/M phase of cell cycle, causes apoptotic cell death by activating caspases, and induces oxidative stress both in Detroit 562 and FaDu cells in a concentration- and a time-dependent manner. In detailed mechanistic in vitro studies, GSE increases the phosphorylation of H2AX (Ser139) and activates DNA damage checkpoint cascade including ATM/ATR-Chk1/2. GSE treatment also increases intracellular reactive oxygen species (ROS) levels in both Detroit 562 and FaDu cells. We also observed that pretreatment of the thiol antioxidant N-acetyl cysteine (NAC) inhibits intracellular ROS levels thereby, reversing the effect of GSE in both HNSCC cells. In in vivo xenograft studies using both Detroit 562 and FaDu cells, GSE feeding decreased the tumor volume by 67% and 65% ($p<0.001$), respectively. Similarly, tumor weight in GSE fed group was decreased by approximately 70 %. Tumor tissue analysis revealed that GSE decreases the expression of biomarker for proliferation (PCNA) but increases the expression of biomarkers for apoptosis (TUNEL) and DNA damage (phospho-H2A.X). Our results obtained from in vitro and in vivo studies demonstrate that GSE could be an effective agent against HNSCC.
Sangeeta Shrotriya, 330-550-7291, Sangeeta.Shrotriya@UCDenver.edu

POSTER # 33

INDOLEQUINONE MECHANISM-BASED INHIBITORS OF QUINONE REDUCTASES: STRUCTURAL BASIS FOR SELECTIVE INHIBITION OF NQO2 RELATIVE TO NQO1.

Chao Yan[1], Marine Dufour[2], David Siegel[1], Philip Reigan[1], Joe Gomez[1], Biehuoy Shieh[1], Christopher J. Moody[2], David Ross[1].
[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver; [2]School of Chemistry, University of Nottingham, Nottingham, UK.

There are two quinone reductases that occur in mammalian systems NAD(P)H:quinone oxidoreductase 1 (NQO1, EC 1.6.99.2) and NRH:quinone oxidoreductase 2 (NQO2, EC 1.10.99.2). NQO1 has been shown to be up-regulated in many types of human solid cancer; whereas NQO2 has been shown to be highly expressed in leukemias. In order to explore their potential as therapeutic targets, we have developed potent and selective mechanism-based inhibitors for both enzymes centered on the indolequinone pharmacophore. Previously, we characterized ES936 (5-methoxy-1,2-dimethyl-3-(4-nitrophenoxy)methylindole-4,7-dione) as an efficient mechanism-based inhibitor of NQO1 and we have now developed a series of indolequinones as mechanism-based inhibitors of NQO2. Substitution of the 5-methoxy group in ES936 with an aminoalkylamino side chain generated potent mechanism-based inhibitors of NQO2 that did not efficiently inhibit NQO1. NQO2 inhibition was observed with compounds with 5'-aminoalkyl chains that varied from 3 to 8 carbons in length, with maximal inhibition observed with compounds that possessed 5'-chain lengths of between 3 to 5 carbons. Compounds with substitutions at the 6-position were not efficient mechanism-based inhibitors of NQO2. As previously observed for a series of NQO1 mechanism-based inhibitors, structural requirements for efficient inhibition of NQO2 also included a substituent at the 3-position capable of leaving to generate a reactive iminium electrophile following enzymatic reduction. Selected indolequinones were tested for NQO2 inhibition using the human leukemia cell line K562 and were found to inhibit NQO2 activity in K562 cells at nanomolar concentrations while not affecting NQO1 activity. Computational-based molecular modeling simulations demonstrated the substantial difference between the active site of NQO1 and NQO2 and ESI-LC/MS provided possible explanations for selective enzyme inhibition. These indolequinones represent the first mechanism based inhibitors of NQO2 to be characterized.
David Siegel, 303-724-3518, david.siegel@ucdenver.edu

POSTER # 34

ACUTE COBALT TOXICITY TO DUGESIA DOROTOCEPHALA

A. Sternenberger, O.R. Arnold, H.S. Ramsdell,
Colorado State University / Center for Environmental Medicine

Dugesia dorotocephala is a useful invertebrate species for aquatic toxicity testing. This free-living flatworm is easy to culture and their size facilitates convenient observation and makes tissue residue analysis feasible. These studies were intended to define the lethal concentration of a soluble cobalt salt (CoCl2) for D. dorotocephala and to identify sublethal effects that may be useful endpoints for chronic tests. Planaria from laboratory cultures were exposed to dilutions prepared with moderately hard reconstituted water in 10 cm diameter glass dishes. Five planaria were added to each dish along with 50 ml of exposure solution with nominal concentrations expressed as mg Co/L. Test solutions were renewed every two days. Observations included mortality, fissioning (the normal reproductive mode of the planaria used in these studies), motility and behavioral signs. In a range-finding study, complete mortality was observed after 6 days at a concentration of 62.5 mg/L whereas no mortality resulted in the 12.5 mg/L treatment. Both the control and 0.1 mg/L groups exhibited a fissioning rate of 20% within 6 days but no fissioning was observed after exposure for 25 days to 2.5 mg/L Co or greater. These observations suggest that suppression of fissioning may be a useful indicator of sublethal stress due to Co exposure. In another study using a range of Co concentrations from 12.5 to 62.5 mg/L, the median lethal concentration for a six day exposure was 50 mg/L. As in the previous study, Co exposure suppressed fissioning. In both studies, Co exposure resulted in diminished motility and obvious behavioral abnormalities at concentrations as low as 2.5 mg/L. Chronic studies of Co toxicity in D. dorotocephala will utilize fissioning and behavioral end points to determine environmentally relevant toxic effects of cobalt.
Andrea Sternenberger, 303-906-7284, creedlady217@gmail.com

POSTER # 35

STRUCTURALLY DIVERSE CATIONIC NEUROTOXICANTS ATTENUATE EXTRACELLULAR-DEPENDENT CALCIUM SIGNALING IN ASTROCYTES.

Karin Streifel, Albert Gonzales, Briana Trout, Logan Maxwell, Brianne Mohl, Scott Earley and Ronald Tjalkens.
Center for Environmental Medicine, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA

Calcium signaling throughout networks of astrocytes is initiated by synaptic activity in order to increase regional cerebral blood flow (rCBF) in response to the local demand for oxygen and glucose. This increase in intracellular calcium [Ca2+]i, in perivascular astrocytes causes a release of several vasoactive factors from cellular end feet contacting arterioles that causes a rapid dilation of the vessel. Deprecations in rCBF are well described in patients with Parkinson's disease (PD) among other neurodegenerative disease but the mechanisms underlying these decreases are unknown. To examine the possible contribution of astrocyte dysfunction to this phenomenon, we postulated that several structurally diverse neurotoxicants of the basal midbrain, all of which are cationic, would inhibit transmitter-induced calcium signaling in culture astrocytes: MPP+, the active metabolite of the model parkinsonian neurotoxicant, 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP); Paraquat (PQ); 6-Hydroxydopamine (6-OHDA); and Manganese (Mn2+). Using calcium imaging in primary cultured striatal astrocytes, we investigated the effect of acute treatment with each neurotoxicant on agonist-induced intracellular calcium transients. We observed a dose dependent decrease in ATP-induced [Ca2+]i transients with acute application of MPP+, 6-OHDA, PQ and Mn2+. A similar observations of diminished [Ca2+]i signaling was noted with OAG, a TRPC channel agonist. These findings indicate that endogenous and exogenous chemicals that are structurally diverse but that have cationic properties inhibit physiological calcium signaling in astrocytes. Because these astrocytic signals are critical to regulation of rCBF, these data suggest a new target for neurotoxicants that may provide insight into mechanisms of decreased cerebral blood flow in PD.
Karin Streifel, 720-565-0217, karin.streifel@gmail.com

POSTER # 36

HIGH-THROUGHPUT SCREENING OF THE NTP MANDATED ICCVAM/ NICEATM CHEMICAL LIBRARY FOR ANDROGEN RECEPTOR ENDOCRINE DISRUPTORS

Todd A. Thompson, Megan K. Dennis, Debra A. MacKenzie, Andrei Leitao, Larry A. Sklar, Eric R. Prossnitz.
University of New Mexico Health Sciences Center, Albuquerque, NM.

Exposure to environmental chemicals may promote processes that contribute to prostate cancer, a disease that accounts for a quarter of all cancers diagnosed among men in the United States. The Multifunctional Androgen Receptor Screening (MARS) assay was developed as a high-throughput, flow cytometry-based assay to assess the properties of compounds that modulate activation of the androgen receptor (AR), a critical contributor to prostate cancer development and progression (Dennis et al., Cytometry A, 2008). The MARS assay utilizes androgen-independent human prostate cancer-derived PC3 cells that are transfected to co-express the wild-type human AR and an androgen-sensitive promoter regulating the expression of destabilized EGFP. The assay is formatted for high-throughput screening using the HyperCytÒ auto-sampling system and flow cytometry (Edwards et al., Methods Mol. Biol., 2009). A chemical library of known and putative endocrine active substances was recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to facilitate the validation of in vitro endocrine disruptor assays. The ICCVAM/ NICEATM chemical library was used to validate the utility of the MARS assay resulting in the identification of compounds with a range of activities on AR transcription. The endogenous AR ligand dihydrotestosterone and the therapeutic AR antagonists bicalutamide and cyproterone acetate performed as anticipated. Interestingly, both bicalutamide and cyproterone acetate showed AR agonist activity at higher concentrations. Analysis of screening results from the ICCVAM/ NICEATM library indicated that 19 compounds exhibited AR agonist activities, 37 chemicals were antagonists, 16 compounds displayed partial agonist characteristics, while the remaining agents showed no activity. Dose-response analyses of ICCVAM/ NICEATM substances were performed to determine the potency of novel endocrine disruptor compounds identified through this screening assay. This study demonstrates that the MARS assay is a sensitive, high-throughput assay that can be used to detect novel chemicals with AR endocrine disruptor activity, which will ultimately lead to a better understanding of the mechanism of action of environmental agents that act as endocrine disruptors.

POSTER # 37

SILIBININ MODULATES UVA-INDUCED PHOTOCARCINOGENESIS THROUGH MITOGEN-ACTIVATED PROTEIN KINASE SIGNALING IN HaCaT CELLS

Tilley, Cynthia; Gu, Mallikarjuna; Narayanapillai, Sreekanth; Agarwal, Rajesh.
Department of Pharmaceutical Sciences, University of Colorado Denver.

Exposure to ultraviolet (UV) radiation induces DNA damage, oxidative stress, and inflammation in human skin which leads to melanoma and non-melanoma skin cancer (NMSC). Ultraviolet A (UVA) is a potent inducer of reactive oxygen species which can induce various biological damages including apoptosis. Earlier studies by us have suggested the strong efficacy of the natural agent 'silibinin' against ultraviolet B (UVB) photocarcinogenesis both in vitro and in vivo. However, modulating effects of silibinin against UVA-induced skin carcinogenesis is not known. Here, we focus on UVA-mediated activation of mitogen-activated protein kinase (MAPK) pathways and their role in inducing activator protein-1(AP-1) and NF-κB mediated transcription. Our completed studies showed that 250 kJ/m2 of UVA induces p38 phosphorylation in HaCaT cells. Pre- and/or post- treatment of silibinin (50, 75 and 100μM) inhibited the p38 phosphorylation in a dose-dependent manner. The activation of ERK was through the combined effect of UVA and silibinin, while JNK levels were not altered. Since MAPKs, specifically p38, appear to play a major role in the UVA-induced photocarcinogenesis; targeting p38 along with other MAPKs by silibinin will aid in the development of effective skin cancer prevention strategies.
Cynthia Tilley, (915)873-1487, cynthia.tilley@ucdenver.edu

POSTER # 38

GENETIC AND PHARMACOLOGIC BLOCKADE OF NF-KB PREVENTS ONGOING NEUROINFLAMMATORY INJURY IN THE MPTP MODEL OF PARKINSON'S DISEASE

Briana R. Trout1, James A. Miller1, Stephen Safe2, and Ronald B. Tjalkens1
1Center for Environmental Medicine, Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, USA
2Institute of Bioscience and Technology, Texas A&M Health Science Center, Houston, TX, USA

Inflammatory activation of glia is implicated in the progressive loss of dopaminergic neurons in Parkinson's Disease (PD). Suppression of neuroinflammation may prove useful in slowing continuous neuronal degeneration. In the present study we set out to investigate the efficacy of nuclear factor kappa B (NF-kB) inhibition in attenuating progressive dopamine neuron loss through both genetic blockade and pharmacological intervention. First, we established a model of progressive neurodegeneration/neuroinflammation employing MPTP in conjunction with probenecid in transgenic NF-kB-EGFP reporter mice. Animals were treated every other day for 7 days with MPTP (20 mg/Kg) and Probenicid (250 mg/Kg) and monitored for a total of 14 days. To assess loss of dopaminergic neurons, stereological counts of TH positive neurons in the SNpc were determined on day 7 and day 14. Stereological assessment revealed a reduction in the total number of dopaminergic neurons on day 7 which progressed to an even greater loss on day 14. These data show that upon cessation of MPTP/probenecid treatment, loss of nigral neurons continues to occur, emulating the progressive nature of the lesion in PD. In addition, robust activation of astrocytes and microglia was observed that correlated with increased activation of NF-kB. Using this model, we assessed the efficacy of novel para-substituted diindolylmethane (cDIM) compounds in attenuating continual neuron loss in vivo. cDIM5 (1,1-bis(3'-indolyl)-1-(p-methoxy)- methane), which induces downregulation of prototypic neuroinflammatory gene NOS2 in primary astrocyte cultures, was given via oral gavage (50 mg/kg) once daily to mice following 7 days of MPTP treatment. Stereological assessment revealed a significant attenuation of dopamine neuron loss in animals treated with cDIM5, as well as decreased glial activation. This process was repeated using cDIM12 (1,1-bis(3'-indolyl)-1-(p-chlorophenyl)- methane), a Nurr1 agonist, that also attenuated dopamine neuron loss. Finally, we evaluated the affect of genetically altering NF-kB signaling in astrocytes on dopamine neuron survival in mice treated with MPTP and probenecid. Mice deficient in astrocytic IKKβ treated using the progressive MPTP and probenecid model displayed significantly less dopamine neuron loss than their wild type counterparts subjected to the same treatment. These results suggest that NF-kB is an important pathway mediating neuroinflammatory activation of astrocytes leading to loass of dopamine neurons and that interdicting this pathway may be a viable therapeutic option for slowing the progression of PD.
Briana Trout, 970-491-2771, brianat@rams.colostate.edu

POSTER # 39

QUINONE-INDUCED PROTEASOMAL INHIBITION AND THE RELATIONSHIP OF PROTEASOME INHIBITION TO CHANGES IN OTHER CELLULAR PROTEIN HANDLING SYSTEMS

Rui Xiong, David Siegel, David Ross
Department of Pharmaceutical Sciences, School of Pharmacy, Anschutz Medical Center, University of Colorado Denver, Denver, CO.

Toxic quinone metabolites derived from dopamine have been implicated in the pathogenesis of Parkinson's disease (PD), which is characterized by degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta. Previously, we demonstrated that dopamine-derived quinones inhibited the proteasome and further triggered apoptosis in dopaminergic cells. In the current study, we examined whether 1,4-benzoquinone (BQ), menadione (MD) and the dopamine-derived quinone aminochrome (AC) could inhibit proteasomal activity in an immortalized mesencephalic dopaminergic neuronal cell line (N27 cell line). Our data showed that both BQ and MD could inhibit proteasome activity in a dose- and time-dependent manner. However, AC had only a moderate effect on proteasome activity. To better characterize biochemical changes after proteasome inhibition, we utilized the proteasome inhibitor MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) and examined several major protein handling systems, including the ER stress/ unfolded protein response (UPR), autophagy, the heat shock protein response (HSPs) and aggresome formation. Results from preliminary experiments showed that MG132-induced proteasome inhibition resulted in stimulation of the UPR and autophagic flux in the early phase (up to 6 hours after proteasomal inhibition) as determined by upregulated expression of phosphorylation of the eukaryotic translation initiation factor 2alpha (eIF2α), C/EBP homologous protein (CHOP) and turnover of autophagy related microtubule-associated protein 1 light chain 3 (LC3). Moreover, after prolonged proteasome inhibition induced by MG132 (> 6 hours) we observed increased cellular level of HSP70, the formation of vimentin-caged aggresomes and apoptosis. Our data suggests a potential link between proteasome functionality and activation of other protein handling systems. These data also suggest that the mechanisms of induction of these alternate protein handling systems and their temporal relationship may be important parameters determining the extent of accumulation of misfolded proteins in cells as a result of proteasome inhibition. This work was supported by NIH grant R01ES018943.
Rui Xiong, 720-273-7431, Rui.Xiong@ucdenver.edu

POSTER # 40

DEVELOPMENT OF NOVEL INDOLEQUINONE ANTITUMOR AGENTS: MECHANISM OF TOXICITY IN HUMAN PANCREATIC CANCER.

Chao Yan[1], David Siegel[1], Marie Colucci[2], Aurelie Chilloux[2], Jeffery Newsome[2], Christopher J. Moody[2], David Ross[1].
[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Colorado Denver; [2]School of Chemistry, University of Nottingham, Nottingham, UK.

A series of novel indolequinones was developed as potent antitumor agents against human pancreatic cancer. Three general classes of indolequinones with varying substitutions at the indole 2- position exhibited marked growth inhibitory activity against human pancreatic cancer both in-vitro (MTT and clonogenic assays) and in-vivo (mouse xenograft models). The pancreatic cancer cell lines PANC-1, MIA PaCa-2, and BxPC-3 were used as in-vitro model systems and the IC50 values of the indolequinones in all three cell lines were in the low nanomolar range. Indolequinones were also found to be efficient inducers of apoptosis in these cell lines at concentrations which induced growth inhibition. Selected indolequinones were screened against the NCI-60 cell line panel and their spectrum of activity was similar to established inhibitors of thioredoxin reductase. Indolequinones were therefore tested as potential inhibitors of thioredoxin reductase and were found to inhibit the enzyme in pancreatic cancer cells at concentrations equivalent to those inducing growth inhibitory effects. The mechanism of inhibition of thioredoxin reductase by the indolequinones was then studied in detail in cell-free systems using purified enzyme. The C-terminal selenocysteine of thioredoxin reductase was characterized as the primary adduction site of the indolequinone-derived reactive iminium using LC-MS/MS analysis. Inhibition of thioredoxin reductase by indolequinones in pancreatic cancer cells resulted in a shift of thioredoxin redox state to the oxidized form and activation of the p38/JNK signaling pathway. Oxidized thioredoxin is known to activate apoptosis signal-regulating kinase 1 (ASK1), the upstream activator of p38/JNK in the MAPK signaling cascade, and this was confirmed in our study providing a potential mechanism for indolequinone-induced apoptosis. Our results describe the redox and signaling events involved in the mechanism of growth inhibition induced by novel inhibition of thioredoxin reductase in pancreatic cancer cells.
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Chao Yan, 303-724-3518, chao.yan@ucdenver.edu

POSTER # 41

CHK1 AND PARP-1 IN THE DNA DAMAGE RESPONSE INDUCED BY TGHQ IN HUMAN PROMYELOCYTIC LEUKEMIA CELL LINE HL-60

Fengjiao Zhang[1], Serrine S. Lau[1], and Terrence J. Monks [1]*
[1] Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona Health Science Center, Tucson, AZ 85721

2,3,5-tris(glutathion-S-yl)hydroquinone (TGHQ), a metabolite of benzene, stimulates the rapid formation of reactive oxygen species (ROS) in HL-60 cells which play a causative role in TGHQ-induced HL-60 cell apoptosis. Since ROS are highly reactive and capable of producing DNA damage, we investigated the effects of TGHQ on DNA-damage-response pathways in HL-60 cells. TGHQ induced severe DNA damage evidenced by a marked DNA ladder formation and H2AX phosphorylation. In addition, the DNA damage initiated the cell cycle checkpoint response, with the activation of both Chk1 and Chk2, and the subsequent degradation of Cdc25A phosphatase, revealing that TGHQ induces cell cycle arrest in HL-60 cells. FACS analysis further verified TGHQ's ability to promote Chk1-dependent S-phase arrest in HL-60 cells. Following S-phase cell synchronization with aphidicolin, TGHQ reduced Chk1 protein levels and inhibited Chk-1 S345 phosphorylation which might combine to drive cells into premature mitosis in the presence of un-repaired DNA damage. However, TGHQ-induced DNA damage also resulted in the activation of the DNA nick sensor enzyme, PARP-1, leading to ATP depletion. Interestingly, both PARP-1 and caspase activation are involved in TGHQ-induced HL-60 cell apoptosis. Thus a total caspase inhibitor (z-vad-fmk) abrogated apoptosis, and PJ-34 (a PARP-1 inhibitor) cooperated with z-vad-fmk to further decrease TGHQ-induced apoptosis. These findings suggest that PARP might participate in caspase activation. Indeed, PARP-1 activation appears to contribute to TGHQ-induced capase-3, caspase–7, and caspase-9 activation. Finally, TGHQ-induced, PARP-dependent cell death was accompanied by the nuclear accumulation of apoptosis-inducing factor (AIF). Thus, PJ34 inhibited TGHQ-induced AIF nuclear translocation, although neither JNK nor p38 MAPK activation appears to be required for AIF translocation. In summary, TGHQ causes Chk1-dependent S-phase arrest in HL-60 cells, and PARP-1 activation contributes to the apoptotic HL-60 cell death at least partially by potentiating the TGHQ-induced caspase activation.
Fengjiao Zhang, 520-306-7581, fzhang@pharmacy.arizona.edu

POSTER # 42

ALL-TRANS-RETINOIC ACID AFFORDS CYTOPROTECTION AGAINST REACTIVE OXYGEN SPECIES-INDUCED RENAL INJURY. Sapiro, J.M., Lord-Garcia, J.L., Jia Z, Gallegos, A.G., Canatsey, R.D., Monks, T.J., and Lau, S.S. Southwest Environmental Health Sciences Center, Dept of Pharm/Toxicol., College of Pharmacy, University of Arizona, Tucson, AZ.

Ischemia reperfusion injury (IRI) and chemical-induced nephrotoxicity are major etiologies of acute kidney injury during which reactive oxygen species are released. 11-Deoxy-16,16-dimethyl-prostaglandin E_2 (DDM-PGE_2) protects against 2,3,5-tris-(glutathion-*S*-yl)hydroquinone (TGHQ) induced, ROS-dependent cell death in LLC-PK_1 cells. Immunoblotting and proteomics analyses revealed that DDM-PGE_2 cytoprotection was associated with a time-dependent increase in retinol binding protein (RBP) synthesis, suggesting that retinoid signaling is engaged during this process. Pharmacological manipulations that abolished the ability of DDM-PGE_2 to induce RBP abrogated its cyto-protective effects, further indicating that RBP is necessary for DDM-PGE_2 mediated cyto-protection. While both all-trans-retinoic acid (ATRA) and 9-cis-retinoic acid (9-cisRA) possess biological activity, pretreatment with ATRA, but not 9-cisRA, afforded cyto-protection in LLC-PK_1 cells following TGHQ treatment. Moreover, the cytoprotective kinetics of ATRA and DDM-PGE_2 were identical, with maximal RBP induction and cyto-protection occurring at 12 and 24 hours, respectively. Chemical hypoxia was established in LLC-PK_1 cells to recapitulate IRI conditions by exposing cells to 0.1 or 1 μM antimycin A for 4 hours in glucose free media. Consistent with the effects of ATRA on TGHQ-induced cytotoxicity, ATRA pretreatment completely protected hypoxic cells from cyto-toxicity as assessed by a mitochondrial dehydrogenase enzyme activity assay (MTT). Moreover, ATRA significantly reduced 8-oxo-deoxyguanosine levels in human kidney HK2 cells after TGHQ challenge. Collectively, these data reveal that ATRA protects renal cell injury, at least in part, *via* suppression of ROS-mediated oxidative damage. Therefore, ATRA may provide an effective therapeutic strategy in chemical-induced renal injury or pathological conditions where ROS contribute to the disease progression. (ES006694, ES016578).

POSTER

Jessica Sapiro, 520-626-9905, sapiro@pharmacy.arizona.edu

	Last	First	University Affiliation	Email
1	Arnold	Olivia	Colorado State University	olivia.arnold@colostate.edu
2	Boitano	Scott	University of Arizona	sboitano@email.arizona.edu
3	Bolt	Alicia	University of Arizona	bolt@pharmacy.arizona.edu
4	Berkbigler	Jennifer	Colorado State University	jberkbig@rams.colorstate.edu
5	Burchiel	Scott	University of New Mexico	sburchiel@salud.unm.edu
6	Canet	Mark	University of Arizona	canet@pharmacy.arizona.edu
7	Chandler	Joshua	University of Colorado Denver	joshua.chandler@ucdenver.edu
8	Chen	Ying	University of Colorado Denver	ying.chen@ucdenver.edu
9	Collinge	Mark	Pfizer Inc.	mark.collinge@pfizer.com
10	Damback	Donna	Genentech Inc.	dambach.donna@gene.com
11	Derry	Molly	University of Colorado Denver	molly.derry@ucdenver.edu
12	Druwe	Ingrid	University of Arizona	druwe@pharmacy.arizona.edu
13	Everson	Gregory	University of Colorado Denver	greg.everson@ucdenver.edu
14	Fidler	Trevor	University of Utah	Trevor.Fidler@utah.edu
15	Friedman	Jacob	University of Colorado Denver	jacob.friedman@ucdenver.edu
16	Fritz	Kristofer	University of Colorado Denver	kristofer.fritz@ucdenver.edu
17	Galligan	James	University of Colorado Denver	james.galligan@ucdenver.edu
18	Gao	Bin	National Institutes of Health	bgao@mail.nih.gov
19	Gao	Jun	Los Alamos National Laboratory	jgao@lanl.gov
20	Gomez-Rubio	Paulina	University of Arizona	gomezp@email.arizona.edu
21	Gritz	Stephanie	University of Colorado Denver	stephanie.gritz@ucdenver.edu
22	Hardwick	Rhiannon	University of Arizona	hardwick@pharmacy.arizona.edu
23	Herndon	Joe	University of Arizona	herndon@pharmacy.arizona.edu
24	Inturi	Swetha	University of Colorado Denver	swetha.inturi@ucdenver.edu
25	Jackson	Brian	University of Colorado Denver	brian.jackson@ucdenver.edu
26	James	Jana	University of Utah	jana.james@utah.edu
27	Kirkley	Kelly	Colorado State University	kelly.anne.sullivan@colostate.edu
28	Klein	David	University of Arizona	klein@pharmacy.arizona.edu
29	Klimecki	Walt	University of Arizona	klimecki@pharmacy.arizona.edu
30	Koppaka	Vindhya	University of Colorado Denver	vindhya.koppaka@ucdenver.edu
31	Kuhlman	Christopher	University of Arizona	kuhlman@pharmacy.arizona.edu
32	LaBarbera	Daniel	University of Colorado Denver	daniel.labarbera@ucdenver.edu
33	Lau	Serrine	University of Arizona	lau@pharmacy.arizona.edu
34	Lake	April	University of Arizona	lake@pharmacy.arizona.edu
35	Lauer	Fredine	University of New Mexico	flauer@salud.unm.edu
36	LaVelle	James	CDM	lavellejm@cdm.com
37	Liu	KeJian	University of New Mexico	kliu@salud.unm.edu
38	Lizarraga	Lucina	University of Arizona	lizarrag@pharmacy.arizona.edu
39	Mateen	Samiha	University of Colorado Denver	samiha.mateen@ucdenver.edu
40	Matsumoto	Akiko	University of Colorado Denver	akiko.matsumoto@ucdenver.edu
41	McElroy	Cameron	University of Colorado Denver	cameron.mcelroy@ucdenver.edu
42	McPeters	Ryley	Colorado State University	ryley.mcpeters@colostate.edu
43	Miller	James	Colorado State University	james.miller@colostate.edu
44	Monks	Terrance	University of Arizona	monks@pharmacy.arizona.edu
45	Murphy	Brittany	University of Colorado Denver	brittany.murphy@ucdenvr.edu
46	Murphy	Vincent	Array BioPharma	vincent.murphy@arraybiopharma.com
47	Nagy	Amber	Los Alamos National Laboratory	anagy@lanl.gov
48	Reilly	Christopher	University of Utah	chris.reilly@pharm.utah.edu

49	Reilly	Tim	Brisol-Myers Squibb	**timothy.reilly@bms.com**
50	Reed	Matthew	Lovelace Respiratory Research Institute	**mreed@lrri.org**
51	Roberts	Jessica	University of Utah	**jessica.roberts@utah.edu**
52	Ross	David	University of Colorado Denver	**david.ross@ucdenver.edu**
53	Sapiro	Jessica	University of Arizona	**sapiro@pharmacy.arizona.edu**
54	Shaprio	Darien	University of Utah	**darien.shapiro@utah.edu**
55	Sherwood	Cara	University of Arizona	**caras@email.arizona.edu**
56	Shrotriya	Sangeeta	University of Colorado Denver	**sangeeta.shrotriya@ucdenver.edu**
57	Siegel	David	University of Colorado Denver	**david.siegel@ucdenver.edu**
58	Singh	Surendra	University of Colorado Denver	**surendra.singh@ucdenver.edu**
59	Smathers	Rebecca	University of Colorado Denver	**rebecca.smathers@ucdenver.edu**
60	Sternenberger	Andrea	Colorado State University	**creedlady217@gmail.com**
61	Streifel	Kare\in	Colorado State University	**karin.streifel@gmail.com**
62	Thompson	Todd	University of New Mexico	**tthompson@salud.unm.edu**
63	Tilley	Cynthia	University of Colorado Denver	**cynthia.tilley@ucdenver.edu**
64	Trout	Briana	Colorado State University	**brianat@rams.colostate.edu**
65	Wempe	Michael	University of Colorado Denver	**michael.wempe@ucdenver.edu**
66	Weis	Christopher	NIEHS	**christopher.weis@nih.gov**
67	Welch	Kevin	USDA	**kevin.welch@ars.usda.gov**
68	Whitman	Samantha	University of Arizona	**swhitman@email.arizona.edu**
69	Xiong	Rui	University of Colorado Denver	**rui.xiong@ucdenver.edu**
70	Yan	Chao	University of Colorado Denver	**chao.yan@ucdenver.edu**
71	Zimmerman	Michael	University of Colorado Denver	**michael.zimmerman@ucdenver.edu**
72	Zhang	Fengjiao	University of Arizona	**fzhang@pharmacy.arizona.edu**
73	Zhao	Fei	University of Arizona	**zhao@pharmacy.arizona.edu**