ABSTRACTS

2022 OVSOT Annual Meeting

October 14, 2022

University of Louisville

Louisville, KY

Platform Presentations

PD #1
Lexiao Jin, University of Louisville, “Electronic Cigarette-derived Aerosols, Endothelial Dysfunction, and Platelet Activation in Mice: Role of Acrolein and Formaldehyde”

PD #4
Alexandria Nail, University of Louisville, “Chronic Arsenic Exposure Reduces ATM Pathway Activation in Human Keratinocytes”

PD #5
James Wise, University of Louisville, “Effect of N-Acetyltransferase Polymorphism on Metabolism and Genotoxicity of 4,4’-Oxydianiline (ODA)”

PHD #7
Ester Bolatimi, University of Louisville, “Effects of subchronic dietary zinc supplementation on high fat diet-induced non-alcoholic fatty liver disease”

PHD #9
Mariam, Habil, University of Louisville, “Double-edged sword nature of N-acetyltransferase polymorphisms”

PhD #18
Kennedy Walls, University of Louisville, “Hepatic metabolism of heterocyclic amines contributes to induction of glucose production and gluconeogenic gene expression in hepatocytes”

Big Picture Science Presentations

PHD #11
Luke Lu, Purdue University, “Novel Discovery of Copper in Modulating Neurogenesis in Adult Brain”

PHD #13
Idoia Meaza, University of Louisville, “The Unloading of Cohesin from Chromatin a New Mechanism for Hexavalent Chromium-Induced Carcinogenesis”

PHD #14
Belinda Petri, University of Louisville, “Polychlorinated biphenyls alter hepatic m6A mRNA methylation in a mouse model of environmental liver disease”

PHD #17
Samuel Vielee, University of Louisville, “A Mechanistic Approach to Categorizing Cr(VI) as a Gerontogen Using a Toxic Aging Coin”

PHD #19
Catlin Wilkerson, University of Louisville, “Alcohol Activates Dgat2 through α4 Nicotinic Cholinergic Receptors in Hepatocyte Models”

MS #1
Krishna Awasthi, Wright State University,” Impact of Topical Gemcitabine treatment on Human and Murine Skin”

UG #5
Afi Tagnedji, University of Louisville, “Upregulation of Cytidine Deaminase in NAT1 Knockout Breast Cancer Cells”

High School Student Abstracts

Abstract # HS 1

Abstract Title

Differences in resistance to erastin induced ferroptosis in the HaCaT and KerCT cell lines are not due to differences in glutathione amounts or metabolism

Authors

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Background: Erastin is a small molecule capable of inducing ferroptosis, a non-apoptotic form of programmed cell death caused by an accumulation of lipid peroxides. It does this by limiting Glutathione (GSH) synthesis. GSH protects cells from ferroptosis by detoxifying lipid peroxides. Previous studies have shown a difference in the susceptibility to ferroptosis in the HaCaT and KerCT keratinocyte cell lines; HaCat cells are resistant to erastin-induced ferroptosis while KerCT cells are susceptible. **Hypothesis**: Differences in GSH metabolism between HaCaT and KerCT cells are responsible for their different sensitivities to ferroptosis. **Methods:** HaCaT and KerCT cells were plated in 6-well culture dishes. After culturing overnight, the medium was changed and the cells were treated with 10 μM erastin. After 24 hours, the conditioned media and cells were collected, and components of the GSH metabolic pathway (GSH, oxidized glutathione (GSSG), cysteine (Cys) and cystine (Cyss)) were measured by HPLC. Cellular concentrations were normalized to total protein and measured by the Bio-Rad DC protein assay. **Results:** Without erastin, the 2 cell lines had similar cellular GSH levels. Both cell lines conditioned their media by importing Cyss and exporting Cys, GSH and GSSG, as evidenced by lower concentrations of media Cyss and the appearance of the others after 24 hours in culture. Erastin exposure resulted in a nearly total loss of all measured components of the GSH pathway from both the intracellular and extracellular compartments with the exception of media Cyss, which was higher. **Conclusions:** Our results demonstrate that erastin blocked Cyss import and depleted cellular GSH in both resistant and susceptible keratinocytes. GSSG did not accumulate in either cell line, and neither GSH nor Cys was increased in either compartment, suggesting that oxidation, export and metabolism could be ruled out as the major fate of GSH in erastin-treated cells. Therefore, it is likely that conjugation to electrophilic lipid peroxidation breakdown products accounts for the loss of GSH. In summary, our hypothesis was not supported by the evidence: differences in resistance to ferroptosis in the HaCaT and KerCT cells lines were not due to differences in GSH levels or its metabolism.

Abstract # HS 2

Abstract Title

Exploring PCB-induced cardiotoxicity in three genotypes of mice

Authors
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Polychlorinated biphenyls are ubiquitous persistent organic pollutants found primarily in fatty fish, but exposure can also occur through older buildings where PCBs were used in electrical equipment as well as wood treatments and caulks. Although they were banned decades ago, their complex chemical structure means PCB body burdens remain high along with widespread warnings to avoid consuming fish from contaminated lakes and rivers. To model human exposures, we treated pregnant mice from three genotypes with a mixture of coplanar (PCB77, 126 and 169) and non-coplanar congeners (PCB105, 118, 138, 153 and 180) by gavage at gestational day 10 (GD 10) and postnatal day 5 (PND 5). Controls received the corn oil vehicle. The mice were wild type *AhrbCyp1a2(+/+)* mice with high affinity for coplanar PCBs, *AhrbCyp1a2(-/-)* knockouts and *AhrdCyp1a2(-/-)* knockouts with poor affinity for binding coplanar PCBs. We tested offspring at P60 and found multiple deficits in motor function and learning and memory. Following behavioral experiments, we followed the mice out to 13 months of age to look for physiological changes related to known endocrine-disrupting effects. In this study, we used H&E staining to examine heart tissue from treated and control animals of all three genotypes. We found a significant gene x treatment interaction with PCB-exposed *AhrbCyp1a2(-/-)* knockouts having significantly more cardiomyocytes in the ventricles compared with their corn oil-treated controls ($P < 0.05$). This suggests some level of hypertrophy in the group that was also most susceptible to PCB-induced neurotoxicity.

Undergraduate Student Abstracts

Abstract # UG 1

Abstract Title
The Impact of Pre-conception and Gestational E-Cigarette Exposure on Offspring Behavior

Authors
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The use of electronic nicotine delivery systems to aid in smoking cessation has perpetuated a general misconception that they are a safe alternative for nicotine consumption. A large study analyzing self-reported e-cigarette use among 69,503 women across 38 U.S. states revealed 1.1% of women used e-cigarettes within the last 3 months of pregnancy. Nicotine, a popular constituent in e-cigarettes, crosses the placenta, allowing it to directly act on fetal tissues. Previous animal models have shown that gestational nicotine exposure disrupts neurological development, causing neurobehavioral abnormalities in offspring. This study tests the hypothesis that pre-conceptional and gestational exposure to e-cigarette vapor affects anxiety-like behaviors in adult offspring. Dams were exposed for 1 hour per day to e-cigarette vapor produced by High-dose (5% nicotine) Vuse Golden Tobacco pods (Vape) or to filtered room air (Shams), beginning 4 days prior to mate introductions and continued daily until birth of the litter. Litters were housed by sex at weaning and on postnatal days 61 – 75 were tested for anxiety-like behavior in neohypophagia and open field trials. Pre-conceptional and gestational exposure to High-dose Vuse Golden Tobacco (5% nicotine) e-cigarette pods did not *significantly* impact measured anxiety-like behaviors in adulthood. For both behavior tests, there was a significant sex effect found in fecal boli deposition (*neohypophagia* p= 0.0009, *open field* p= 0.0022). Regardless of exposure, adult males were more likely to defecate than adult females when placed in an anxiogenic environment. Increased fecal boli excretion is associated with an increase in anxiety. This suggests male mice are more affected by anxiety-inducing stimuli and are likely to respond with an increase in defecation. Another significant sex effect was found regarding total ambulation in the open field (p=0.0016) in which females, regardless of exposure, traveled a greater horizontal distance than their male counterparts. Despite some statistical trends, our data do not support our hypothesis that *in utero* e-cigarette exposure alters expression of anxiety-like behavior in adulthood, at least as measured by these tests. Several tissues collected (liver, kidney, cecum, brain) are being analyzed for gene expression and histological differences between groups. Funded by the University of Louisville Bridges to Baccalaureate (ULBB) Program, NIH award R25GM133328. Funding also provided by Center for Integrative Environmental Health Sciences P30ES030283 (States, PI; sub-project PI Neal, Co-I Corbitt) and NIH R15ES028440 (Neal, PI).

Abstract # UG 2

Abstract Title

Dopamine and Serotonin Signaling Following Developmental Benzo[a]pyrene Exposure in *Cyp1a1(-/-)* knockout and wild type mice

Authors
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Benzo[a]pyrene (BaP) is a widespread pollutant that exerts neurotoxic effects on early brain development. Our previous studies have found *Cyp1a2(-/-)* knockout mice to be more susceptible to developmental BaP exposure. More recently, we studied *Cyp1a1(-/-)* knockouts. In this study, we treated pregnant dams with either 10mg/kg/day BaP in corn oil-soaked cereal or the vehicle from gestational day 10 until postnatal day 25. At postnatal day 25, a male and female pup from each litter were randomly selected for behavioral testing. Once all behavioral tests were completed at postnatal day 120, we collected the striatum, hippocampus, prefrontal cortex, and hypothalamus. To quantify neurotransmitter levels in each of the brain regions, we used HPLC with electrochemical detection. This measured dopamine, serotonin, and their metabolites DOPAC and 5HIAA. In the hippocampus, there was a significant main effect of genotype. *Cyp1a1(-/-)* knockout mice had higher DOPAC levels ($P < 0.001$) and dopamine turnover ($P < 0.05$) compared with wild type mice. Knockout mice also had significantly lower 5HIAA levels ($P < 0.001$) and a trend for lower serotonin levels ($P = 0.055$). In the hypothalamus, there was a significant main effect of sex. Females had higher levels of all neurotransmitters and metabolites compared with males ($P < 0.05$). There was a significant gene x treatment x sex interaction ($P < 0.05$) for DOPAC and a trend for gene x treatment x sex interaction for 5HIAA levels ($P = 0.07$). These results suggest that genotype and sex have greater influence on neurotransmitter levels than developmental BaP exposure.

Abstract # UG 3

Abstract Title

Impact of Developmental E-cig Exposure Nicotine Levels on Offspring Number, Birth Weight, and Kidney Weights

Authors
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The impact of maternal smoking during pregnancy is well-studied with clear and compelling evidence of increased risk of injury to both the mother and to the fetus. Current harm prevention and risk reduction strategies focus on maternal smoking cessation, with many pregnant women switching to vaping as a perceived 'healthier' way of ingesting nicotine. Nicotine alone is toxic to both the mother and the fetus, yet the reproductive and developmental toxicity of nicotine containing e-cigarette vapor remains poorly characterized. In the current study, we developed murine models of developmental vaping exposure utilizing the commercially available Vuse Golden Tobacco pods with either 1.8 or 5% nicotine. Female mice were exposed to vaping for 1 hr/day starting at 4 days prior to mating and continuing until GD19. At birth, offspring number and general health metrics were collected with offspring monitored until two months of age. Key outcomes noted include a reduction in litter size in both nicotine concentration groups, with higher dose having a larger effect. Birth weight and growth trajectory were not impacted. At the highest dosage (5% nicotine), developmental vaping resulted in a reduction in kidney weights, not seen at the lower dose, indicating dose response of nicotine on this measure. Histological assessment of kidney and liver as well as gene expression in key metabolic pathways is ongoing.

Funding: P30 ES030283 (States, PI), R15 ES028440 (Neal, PI), CHD Summer Bridge Program (Kidd & Corbitt, PIs), and ULBB Program R25GM133328 (Kakar, PI)

Abstract # UG 4

Abstract Title

The Effect of Benzo[a]pyrene on Learning and Memory in *Cyp1a1(-/-)* Knockout and Wild Type Mice

Authors

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Benzo[a]pyrene(BaP) is a polycyclic aryl hydrocarbon that is the result of incomplete combustion in grilled food, cigarette smoke, and car fuel emissions. This widespread pollutant is a carcinogen that can affect motor, neurological, and memory in humans that have been exposed to BaP during early brain development. Our studies were designed to determine if genetic differences affecting BaP metabolism could affect susceptibility to developmental BaP neurotoxicity. Pregnant *Cyp1a1(-/-)* knockout and Cyp1a1(+/+) wild type mice were treated with 10mg/kg/day benzo[a]pyrene BaP) from gestational day 10 to postnatal day 25. One male and one female from each litter were tested starting at P60 using Morris water maze to assess spatial learning and memory. BaP-exposed mice had longer path lengths on all 6 days of testing in the Morris water maze, but the differences were not significant. There was a significant gene x treatment interaction in the Shift-reduced Probe trial with BaP-exposed *Cyp1a1(+/+)* knockout mice having more zone crossings than all other groups ($P < 0.01$). There were no sex differences.

Abstract # UG 5

Abstract Title
Upregulation of Cytidine Deaminase in NAT1 Knockout Breast Cancer Cells

Authors
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Arylamine *N*-acetyltransferase 1 (NAT1) is a polymorphic drug-metabolizing enzyme that participates in detoxification and bioactivation of arylamines, arylhydrazines and similar compounds. In recent years, it was discovered that **NAT1 is upregulated in breast cancer**. However, how NAT1 contributes to breast cancer development and progression remains unclear. To develop novel hypotheses, NAT1 knockout (KO) cell lines (KO2 and KO5) were created from MDA-MB-231 (a triple-negative breast cancer cell line) using CRISPR/Cas9 technology.

According to our proteomics and RNAseq analyses, ***NAT1* KO cells show increased expression of cytidine deaminase (CDA)**, a player in the pyrimidine salvage pathway. Metabolomics data suggested *NAT1* KO cells had **defects in the *de novo* pyrimidine pathway**, which can explain the upregulation of the salvage pathway. Pyrimidine *de novo* synthesis and salvage pathways are essential for DNA and RNA synthesis and cell growth.

We hypothesized that NAT1 KO MDA-MB-231 cells show differential sensitivity to drugs that either inhibit cellular pyrimidine homeostasis or are metabolized by CDA. The cells were treated with 1) inhibitors of dihydro-orotate dehydrogenase or CDA (e.g., teriflunomide and tetrahydrouridine) and 2) naturally occurring, modified cytidines (e.g., 5-formyl-2'-deoxycytidine; 5fdC).

To compare the sensitivity of the parental and two *NAT1* KO MDA-MB-231 cells (KO2 and KO5) to inhibitors of pyrimidine biosynthesis or salvage pathway; and to naturally occurring, modified cytidines the cells were treated with the indicated concentrations of teriflunomide and leflunomide (inhibitors of dihydro-orotate dehydrogenase) or 5fdC (5-formyl-2'-deoxycytidine) or 5hmdC (5-hydroxymethyl-2'-deoxycytidine) for 7 days. 3 days for the former class of drugs. Following the treatment**, cell viability was measured using alamarBlue assay and expressed as 'relative cell viability'** (relative to the untreated control group).We observed the following

- compared to the parental cells, *NAT1* KO cells were more resistant to inhibitors dihydro-orotate dehydrogenase and CDA. *NAT1* KO cells were markedly more sensitive to 5fdC which induces DNA damage in the presence of high CDA activity.
- Co-treatment with 5fdC and a CDA inhibitor, tetrahydrouridine, abrogated 5fdC cytotoxicity in *NAT1* KO cells, indicating that the increased sensitivity of *NAT1* KO cells to 5fdC is dependent on their increased CDA activity.
- The cellular role of NAT1 may be linked to pyrimidine homeostasis in breast cancer cells
- Inhibition or deletion of *NAT1* and subsequent upregulation of CDA may confer increased sensitivity to selective chemotherapeutic drugs.

Abstract # UG 6

Abstract Title

The Effect of Exercise on Anxiety in Mice Developmentally Exposed to Benzo[a]pyrene

Authors

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Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon and known carcinogen with neurotoxic properties with potential impacts on stress and anxiety-like behaviors. Exercise increases endorphin production, which can reduce anxiety. Our previous studies revealed that *Cyp1* knockout mice were more susceptible to developmental BaP neurotoxicity. Our current studies are looking at potential interventions to ameliorate those adverse effects. There were three levels of treatment in addition to controls: dam-only exercise, offspring-only exercise, and dam + pup exercise. Dams had free access to running wheels for 1h/day for two weeks prior to mating through gestational day 10. Their offspring exercised from postnatal day 30 to 60, a time of final brain maturation. We measured anxiety-like behavior using the marble burying test and zero maze. We found a highly significant effect of exercise in the zero maze test when pups exercised. The pup-only exercise and pup + dam group had significantly more head dips and more zone crossings, indicating high exploratory activity ($P < 0.001$). Dam exercise had greater effect in the marble burying test with the dam-only and pup + dam groups burying fewer marbles ($P = 0.057$), an indicator of less anxiety-like behavior. Together, these data suggest both material and offspring exercise can have persistent effects on behavior.

Abstract # UG 7

Abstract Title

The Effect of Prenatal Benzo[a]pyrene Exposure on Motor Functions in *Cyp1a1(-/-)* Knockout and Wild Type Mice

Authors

Suzie Martin, Alex Walsh, Briannia Quarles, Kalyani Abbaraju, Mickayla Kowalski, Connor Perry, Angela Kyntchev, India Davis, Karlee Migneault, Mackenzie Feltner, and Christine Perdan Curran

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Benzo[a]pyrene is a common pollutant found in traffic pollution, grilled foods, and cigarette smoke. These are common exposures in day to day life and have previously been connected to IQ deficits in humans. In order to identify genetic differences that increase risk, we used a mouse model with allelic differences in CYP1A1, a key enzyme used to metabolize BaP. Wildtype and *Cyp1a1(-/-)* knockout dams were either treated with corn-oil soaked cereal (control) or 10mg/kg BaP in corn oil. We tested the developmental of neonatal reflexes using the surface righting reflex test at postnatal days 5, 7 and 10 and the negative geotaxis test at P7, 10 and 14. Once offspring reached 60 days of age, they completed a comprehensive neurobehavioral battery that included the rotarod test of motor coordination and motor learning. There was a trend for a gene x treatment interaction in righting reflex with BaP-exposed knockouts having shorter latencies on P7 and P10 ($P < 0.1$). There was a gene x treatment interaction in negative geotaxis with BaP-exposed wild type mice showing impairments at P10 and P14 compared with corn oil controls. Knockout mice had significantly shorter latencies on P7 ($P < 0.01$). There was a main effect of genotype in the Rotarod test with *Cyp1a1(-/-)* knockout mice having significant impairments compared with wild type mice on Days 2-5 of testing ($P < 0.001$). These data suggest both genotypes of mice are affected by BaP exposure and that CYP1A1 might have a normal function in brain regions required for motor coordination and balance.

Master Student Abstracts

Abstract # MS 1

Abstract Title

Impact of Topical Gemcitabine treatment on Human and Murine Skin

Authors

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Affiliation and Address(es)

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Chemotherapy has remained the mainstay for the treatment of multiple types of cancers. In particular, topical use of chemotherapy has been used for skin cancers. Though effective, topical chemotherapy has been limited due to adverse effects such as local and even systemic toxicities. Our published studies demonstrated that exposure to pro-oxidative stressors, including therapeutic agents induces the generation of extracellular vesicles known as microvesicle particles (MVP) which are dependent on activation of the Platelet-activating factor-receptor (PAFR), a G-protein coupled receptor present on various cell types, and acid sphingomyelinase (aSMase), an enzyme required for MVP biogenesis. Based upon this premise, we tested the hypothesis of whether topical application of gemcitabine will induce MVP generation in human and murine skin. Our *ex vivo* studies using human skin explants demonstrate that gemcitabine treatment results in MVP generation in a dose-dependent manner in a process blocked by PAFR antagonist and aSMase inhibitor. Importantly, gemcitabine-induced MVPs carry PAFR agonists. To confirm the mechanisms, we employed PAFR-expressing and deficient (*Ptafr*$^{-/-}$) mouse models as well as mice deficient in aSMase enzyme (*Spmd1*$^{-/-}$). Similar to the findings using human skin explants, our studies demonstrate that gemcitabine-induced MVP release in WT mice was blunted in *Ptafr*$^{-/-}$ and *Spmd1*$^{-/-}$ mice. These findings demonstrate a possible mechanism by which local chemotherapy can generate bioactive components as a bystander effect in a process that is dependent upon the PAFR-aSMase pathway.

Abstract # MS 2

Abstract Title

Platelet activating factor and microvesicle particles as key mediators of the enhanced inflammatory responses in multiple organs following intoxicated thermal burn injury.

Authors

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Alcohol intoxication prior to burn injury, found in almost half of all hospitalized burn patients, results in increased morbidity and mortality. Numerous studies in murine models have demonstrated that intoxicated thermal burn injury (ITBI) causes an enhanced inflammatory response with increased pro-inflammatory cytokines in multiple organ systems over thermal burn injury (TBI) alone. Though these models have yielded significant insights, the exact mechanism behind these pathologic outcomes remain unknown. Intoxicated thermal burn injury generates high levels of the lipid mediator platelet activating factor (PAF) and release of microvesicle particles (MVPs) from the skin keratinocyte, which we hypothesize are key effectors in the pathology. MVPs are specifically released from keratinocytes in response to PAF Receptor (PAFR) activation caused by excess PAF generated by ITBI. These subcellular particles transport and thus protect the metabolically labile PAF, allowing it to bind to multiple key sites such as the PAFR in the gut, which activates myosin light chain kinase (MLCK) and increases gut permeability. These events allow bacteria to enter the bloodstream and cause sepsis and dysregulated inflammation in multiple organs. Consistent with this novel model, our current studies show that mice lacking PAFR have lower levels of proinflammatory cytokines in multiple organ systems in response to ITBI, demonstrating the role of PAFR in enhanced inflammatory response caused by this clinically relevant combination. These studies provide a potential mechanism and therapeutic approaches for preventing the damage caused by ITBI.

Abstract # MS 3

Abstract Title

Increased Production of Microvesicle Particles in Response to Ultraviolet B Radiation and Solar Stimulated Light via Microvesicle Particle Signaling in a Murine Model of Photosensitivity

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Xeroderma Pigmentosum (XP) is an uncommon genetic condition which is characterized by an enhanced sensitivity to the DNA damaging effect of ultraviolet radiation (UV). The Nucleotide Excision Repair route (NER) uses XPA, a DNA Damage Recognition and Repair factor, to identify and remove aberrant DNA portions. UVB (290-320 nm) radiation found in the sunlight is necessary to produce vitamin D in human, however, in certain pathologic conditions results in photosensitive conditions including erythema and inflammatory responses. Small membrane bound particles, called microvesicle particles (MVPs) are shed from the plasma membrane of keratinocytes in response to various stimuli which includes lipid Platelet activating factor (PAF). Previous studies from our lab have shown that when the XPA deficient model is exposed to UVB radiation, the damaged keratinocytes release excess amount of reactive oxygen species (ROS) and it leads to non-enzymatic formation of oxidized GPC (ox-GCP) with PAF receptor (PAFR) agonistic activity. The PAFR agonist activate the keratinocyte G-couple protein receptor PAFR, which in the presence of enzyme acid sphingomyelinase (aSMase) triggers MVP release which leads to acute inflammation. Therefore, the purpose of the current research is to determine if XPA deficiency increases increased MVP production in response to UVB as well as solar-stimulated light (SSL) via the PAF-R signaling pathway. SSL emits radiations that closely resemble natural sunlight and contain UVA and UVB radiations. When exposed to UVB and SSL radiation, studies using keratinocyte cell lines lacking XPA showed increased MVP release in comparison to XPA-positive cells. Similarly, UVB and SSL treatment of XPA KO mice resulted in increased MVP release, erythema, and cytokine production in comparison to wild-type mice. These effects were blocked by treatment with the aSMase inhibitor imipramine. These studies indicate potential involvement of MVP generation in photosensitivity associated with XPA deficiency and provide novel treatment strategies.

Abstract # MS 4

Abstract Title
Use of Porcine Explants to Model Microvesicle Particle Generation.

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Text of abstract

Recent studies by ours and other groups have indicated an important role of subcellular microvesicle particles (MVP) in the pathologic effects of multiple environmental stressors ranging from burn injury to Ultraviolet B radiation (290-320 nm; UVB). Model systems that have been used have ranged from in vitro cell lines to ex vivo human skin explants to in vivo murine and human systems. However, the lack of readily accessible human skin can be a significant obstacle. Due to the similar physiology of porcine skin to that of human skin and the fact that it is more widely available, we have constructed a skin study model utilizing porcine skin. The goal of these studies is to validate the use of ex vivo skin explants derived from porcine skin. In these studies, we obtained fresh porcine skin and subjected it to agents as varied as topical phorbol ester, Platelet-activating factor agonist, or UVB, with the outcome to measure MVP release from the skin. All of these agents induced MVP release in a similar fashion to human skin explants. Inasmuch as porcine skin can be obtained readily in large amounts, this could be a valuable model to study environmental stressors on skin subcellular particle release.

Abstract # MS 5

Abstract Title
New Approach to Analyzing the Size Distribution of Metallic Aerosols in Welding Fumes

Authors
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Workers are exposed to metallic aerosols produced from manufacturing processes such as welding, smelting, and laser cutting. Identifying the type, quantity, and size of metallic aerosols is essential for assessing the risks of workplace exposures. The industry gold standard for characterizing metallic aerosols involves collecting samples and sending them to third-party labs for metal analysis using inductively coupled plasma (ICP) techniques. However, conventional respirable sampling cannot provide detailed size information and the ICP techniques are expensive and time-consuming. To overcome these limitations, a new approach is introduced in this study. The new method involves collecting aerosols by size using the cascade impactor and analyzing metal contents using field-portable X-ray fluorescence (XRF). To test the new method, area sampling was conducted near the welding stations in a local truck trailer facility. The results were compared to those done using the conventional method to determine the correction factor. The results show the feasibility to overcome the limitation of the conventional methods.

Doctoral Student Abstracts

Abstract # PHD 1

Abstract Title

Effects of long-term exposure to polychlorinated biphenyls on ileal gene expression

Authors

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Background: Polychlorinated biphenyls (PCBs) are persistent organic pollutants manufactured in the 1930s for industrial purposes under the trade name Aroclor (USA). Although PCBs were banned in the 1970s, they are still present in the ecosystem and in living organisms due to their thermodynamic stability and resistance to xenobiotic metabolism. Previously our lab demonstrated sub-chronic PCB exposure resulted in toxicant-associated steatohepatitis (TASH) in a diet-induced obesity model. These observations were in part due to the activation of hepatic nuclear receptors including CAR and PXR, and their ability to regulate gut microbiome composition and intestinal function. However, little is known about the long-term effects of PCBs on ileal and gut microbiome regulation have not been studied. Therefore, this project evaluated the long-term effects of PCBs on ileal gene expression.

Hypothesis: We hypothesize long-term PCB exposure will disrupt tight junction (*Tjp1*) and mucosal barrier integrity genes (*Tff3*) and increase pro-inflammatory markers (*Il-10)* in the intestine.

Methods: Low fat diet fed male C57BL/6J mice were exposed to a single oral gavage of either corn oil (vehicle control, n=10) or Aroclor 1260 (20 mg/kg, n=30) and allowed to age for 34 weeks. Aroclor 1260, a commercial PCB mixture, was selected based on PCB composition reflective of PCB bioaccumulation patterns in humans. Cecum samples were collected for gut microbiome assessment. Intestinal permeability and inflammation markers in Ileum samples were assessed using RT-PCR. Differences in gene expression between PCB and control mice were analyzed using the Mann-Whitney U test at the 0.05 significance level. All statistical analysis was performed on GraphPad Prism (v9.4.1).

Results: We observed an upregulation of Trefoil factor 3 (*Tff3, an* intestinal maintenance/repair of intestinal mucosa) and downregulation of Cadherin 5 (*Cdh5,* an epithelial integrity marker) in mice treated with PCB relative controls. This suggests a disruption of intestinal barrier integrity. However, there were no significant alternations in inflammatory markers.

Conclusion: The preliminary results suggest that long-term PCB exposures affect markers of gut barrier function. Additional ongoing studies examining gut microbiome composition in these mice can help shed more insight into how PCB effects on the gut could contribute to our previously reported PCB-induced TASH.

Abstract # PHD 2

Abstract Title

AhR activation differentially alters the expression profile of IGH isotypes

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a persistent environmental contaminant known to inhibit immunoglobulin (Ig) gene expression and antibody secretion in various animal models. This inhibition is mediated through the aryl hydrocarbon receptor (AhR) for which TCDD is a high-affinity ligand. Most studies evaluating Ig expression have been limited to assessing IgM using animal models. The current study focuses on determining the effects of TCDD and AhR activation on the expression profile of human Ig isotypes (i.e. IgM, IgG1-4, IgA1-2, IgE) utilizing a human Burkitt lymphoma cell line (CL-01) model that can be activated to secrete Ig and undergo class switch recombination from IgM to IgG, IgA or IgE. Our results suggest that TCDD has little to no effect on IgM secretion, but significantly inhibits total IgG secretion, an effect reversed by the AhR antagonist CH223191. The effect of TCDD on the expression of the Ig heavy chain (IGH) constant regions (i.e. μ, γ1-4, α1-2, and ε, which encode for the heavy chain proteins in IgM, IgG1-4, IgA1-2, and IgE, respectively) was also evaluated. At the transcript level, TCDD has little to no effect on μ transcripts but significantly inhibits γ1-4 and ε transcripts. However, α1-2 transcripts increased in response to TCDD. Notably, the AhR antagonist reversed these TCDD-induced effects on IGH expression. Interestingly, the effects of TCDD and AhR antagonist on μ, γ1-4, and ε expression were independent of the AhR having a functional transactivation domain. However, the effect of the AhR antagonist on the expression of α transcripts was greatly altered when the transactivation domain of the AhR was dysfunctional. These results suggest that AhR activation differentially alters the expression profile of IGH isotypes in both a transactivation-dependent and independent manner.

Abstract # PHD 3

Abstract Title**E-cigarette Aerosols Containing Coolants Disrupt Cardiac Autonomic Balance and Evoke Ventricular Premature Beats in Mice**

Authors
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Introduction: Cooling agents can add minty or 'icy' attributes to e-cigarettes and have become increasingly popular. Although these coolants can be toxic in vitro, their toxicity in vivo remains unknown, especially for the synthetic coolants WS-3 and WS-23. To determine the potential for coolants to promote cardiac dysfunction, we tested the electrophysiologic and autonomic effects of e-cig aerosols with varying coolant concentrations.

Methods: Mice (n=8 male C57BL/6J) were exposed to Air (filtered air) or e-cig aerosols from Vehicle (30PG/70VG + 2.5% nicotine benzoate) alone or with Menthol, WS-3, or WS-23. On each exposure day, mice underwent three puff sessions (9-min puffing with 9-min washout) at 0.25% coolant, followed by three sessions at 1% and three sessions at 2.5% coolant. Telemetry-derived electrocardiograms were analyzed for heart rate, heart rate variability (HRV), and arrhythmias by emka ecgAUTO, with $p < 0.05$ by 2-way ANOVA for all reported differences.

Results: Concomitant with 1% and 2.5% coolant puffing, Vehicle significantly decreased heart rate vs. Air. Relative to Vehicle, heart rate decreased during 1% Menthol, whereas it increased during 2.5% WS-23. During the 2.5% coolant puffing phase, Vehicle alone increased RMSSD vs. Air. Relative to Vehicle, RMSSD increased during WS-23 at both 1% and 2.5%, and also during WS-3 at 2.5%. During washouts, 2.5% WS-23 increased heart rate and decreased HRV vs. both Air and Vehicle, suggesting sympathetic dominance. Across the entire 2.5% regimen, WS-23 significantly increased ventricular premature beats relative to both Air and Vehicle, with no other differences.

Conclusions: Specific cooling agents (e.g., WS-23) may dose-dependently enhance the cardiac risks of vaping by promoting sympathetic excitation and arrhythmia. If complemented with human studies, this data may be used by federal regulatory authorities to design and implement tobacco control initiatives that lessen the adverse impacts of vaping on public health.

Abstract # PHD 4

Abstract Title

Validation of inductively coupled plasma-optical emission spectrometry to analyze metals in toenails

Authors

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Welders are exposed to welding fumes containing a high concentration of different metals, some of which are known to be neurotoxic in chronic exposure settings. However, the exact dose-response relationship in humans is not yet fully understood, stressing the importance of an exact assessment of exposure to these different metals. Our group, amongst others, has shown that manganese (Mn) concentration in toenails is a promising biomarker of Mn exposure and body burden, which can be expanded to other metals in welding fumes. Typically, toenail metal levels are being analyzed via inductively coupled plasma (ICP) -mass spectrometry (-MS), a relatively cost-intensive method that provides a low detection limit (>0.001-0.01 ppb). This work explored and validated the use of ICP-optical emission spectrometry (-OES) as an alternative, more cost-efficient analysis method of toenail clippings as a biomarker of metal exposure. ICP-OES was compared to ICP-MS by Pearson Correlation in analyzing toenail clippings from a healthy volunteer for six metals (Mn, iron (Fe), copper (Cu), nickel (Ni), zinc (Zn), and chromium (Cr)). Further, the reproducibility of ICP-OES was tested by repeating the analysis of a sample from the same stock solution three times and the coefficient of variance (CV) was calculated for each metal. Toenail clippings from a non-welder were chosen as test samples because they contain lower concentrations of toenail metals than welders' toenail clippings. Results showed a strong agreement between ICP-OES and ICP-MS results for the six metals (Pearson correlation coefficient = 1.00, 0.78, 1.00, 1.00, 0.84, 0.99 for Mn, Fe, Cu, Ni, Zn, and Cr, respectively). Differences in metal levels in μg/g (%) between ICP-OES and ICP-MS were 0.49 (11%, Mn), 1.14 (4%, Fe), 0.61 (4%, Cu), 0.25 (22%, Ni), 8.41 (3%, Zn), and 0.03 (1%, Cr). The CVs demonstrating reproducibility were 2%, 2%, 3%, 4%, 1%, and 8% for the same metals. These results demonstrate that in the context of measuring metals in toenail clippings of welders as biomarker of exposure, ICP-OES may be used instead of ICP-MS. Next goals include validating this method also for the metal measurement in personal air samples and understanding the intrinsic longitudinal inter- and intra-subject variations of toenail metals by measuring toenail clippings at regular intervals over a longer time. (Supported by NIEHS R01 ES032478 and the International Manganese Institute)

Abstract # PHD 6

Abstract Title
Bacterial Epoxide Hydrolase Gene Markers were Elevated in Human Alcohol-Associated Liver Disease

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Background: Alcohol-associated liver disease (ALD) is a highly prevalent condition resulting from excessive alcohol consumption. Understanding the mechanisms contributing ALD development may help discover potential therapies. Our group recently implicated soluble epoxide hydrolase (sEH) as a pathogenic mediator in ALD and showed that sEH inhibition attenuates experimental ALD in mice. sEH is expressed in nearly all tissues of the body, and proteins with EH activity are widely expressed by gut microbiota. This study aimed to quantify differences in the abundance of gut EHs between healthy controls and individuals with ALD.

Methods: A database of EH-specific gene markers was generated using ShortBRED Identify by inputting a list of proteins with suspected EH activity compiled from NCBI. Differences in microbial EH genes were quantified via the ShortBRED Quantify function between healthy controls (HC, n=8) and those with alcohol use disorder (AUD, n=45) or alcohol-associated hepatitis (AH, n=86) by accessing publicly available shotgun metagenomic data. Intra- and inter-group EH marker family comparisons were made to identify disease-specific markers, which were further linked to their associated gut bacteria.

Results: Compared to HC, individuals with AUD had a significant increase in EH marker abundance ($p<0.01$), which was further increased in AH ($p<0.0001$). Downstream analysis indicated that AUD patients had many EH markers belonging to known gut pathogens including *Salmonella enterica*, among others. Further, AH patients had numerous elevated EH gene markers expressed in bacteria from phyla Bacteroidetes and Firmicutes, including three markers from *Enterococcus faecalis*, a pathogenic microbe previously shown to be elevated in ALD. There were also numerous markers shared between AUD/AH, with little overlap with HC, demonstrating the impact of alcohol use on EH-expressing gut microbes.

Conclusions: Individuals with ALD had increased EH gene abundance, which may indicate that gut EH activity is increased in ALD, which may contribute to pathogenesis of this disease via the gut-liver axis. EH genes elevated in these patients were associated with microbes known to be deleterious in ALD, including the cytolysin-producing *Enterococcus faecalis*, among others. Future work will correlate EH gene markers with disease severity and demographic variables and develop therapeutic strategies to target these microbiota.

Abstract # PHD 7

Abstract Title

Effects of subchronic dietary zinc supplementation on high fat diet-induced non-alcoholic fatty liver disease.

Authors

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Background: Non-alcoholic fatty liver disease (NAFLD) has surpassed alcohol-associated liver disease (ALD) as the most chronic liver disease, affecting approximately 25% of the global adult population. The development of NAFLD and related NAFLD risk factors is associated with zinc deficiency. Clinically, zinc has been used for the treatment of ALD but its therapeutic role in NAFLD remains unclear. Previous studies show reduced hepatic steatosis and improved glucose metabolism with zinc-supplemented high fat diet (HFD); however therapeutic effects of zinc supplementation after established NAFLD are yet to be determined. We therefore developed an *in vivo* model to characterize the effects of zinc supplementation on HFD-induced NAFLD. We hypothesize established NAFLD will be attenuated by dietary zinc supplementation.

Methods: 9-week-old male C57BL/6J mice were fed either a control diet (CD) or HFD (60% fat-kcal) for 12 weeks. After 12 weeks mice were further grouped into diets containing 30 or 90 mg zinc/4057 kcal, representing normal and zinc supplemented diet. Echo MRI and intraperitoneal glucose tolerance tests were performed at weeks 12 and 19, before and after zinc supplementation, respectively. Mice were euthanized at week 20. Plasma and liver tissue were collected for lipid, histology, gene expression and metal analysis.

Results: As expected, 12 weeks of HFD resulted in reduced glucose clearance, increased body weight and percent body fat. Eight weeks of subsequent zinc supplementation did not significantly alter glucose handling nor hepatic gene expression of key regulators of hepatocyte function and lipid metabolism in CD or HFD-fed mice. Hepatic zinc accumulation was not different between normal and zinc supplemented diet groups. Additionally, neither NAFLD histology (steatosis and fibrosis) nor plasma AST and ALT were improved by zinc supplementation in HFD-fed mice.

Conclusions: Results from our model suggest 8-week zinc supplementation cannot reverse established NAFLD. The high fat diet may have caused NAFLD disease progression beyond rescue by zinc supplementation. Alternatively, the 8-week period was not long enough for zinc to have the desired therapeutic effect. Future studies will address these limitations and provide insights to zinc as a therapeutic agent for established NAFLD.

Abstract # PHD 8

Abstract Title

Environmental pollutant, Polychlorinated biphenyl 126, alters energy metabolism in a rodent ALD model

Authors

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Alcohol-associated Liver Disease (ALD) prevalence is rising in the U.S. which is associated with increased hospitalizations, preventable deaths, and economic cost. ALD is characterized by hepatic steatosis, impaired energy metabolism, and hepatocyte injury. Pathologically, exposure to environmental toxicants have also shown to induce similar outcomes but characterized as “Toxicant-associated Fatty Liver Disease” (TAFLD). Previously, environmental toxicant, polychlorinated biphenyl (PCB) 126, has shown to modify or enhance non-alcoholic fatty liver disease in high-fat diet models. However, limited knowledge exists on how other lifestyle related diseases, such as ALD, may be modified or enhanced in conjunction with environmental toxicant exposure.

We hypothesize that PCB126 exposure will enhance steatosis, metabolism disruption, and hepatocyte injury.

Male C57BL/6J mice were exposed to 0.2mg/kg PCB126 or corn oil vehicle by oral gavage. Mice were then fed 5% ethanol or pair fed (0% ethanol) diet for ten days followed by 5g/kg EtOH binge. Two-way ANOVA with Tukey’s multiple comparisons was used for statistical analyses where the alpha level was set to 0.05.

PCB126 activated prototypical transcription factor, *Ahr*, indicated by ~35-fold induction of target gene, *Cyp1a2*. All measures of ethanol-induced steatosis and dyslipidemia (increased liver weight, hepatic triglycerides, and lipid droplet formation) were enhanced in the EtOH+PCB126 group. Gene expression analyses of lipid metabolism related genes indicated increased lipid import and decreased oxidation. Hepatic glycogen content was decreased while gene expression analyses indicate impaired glycolysis and gluconeogenesis. Infiltrating hepatic granulocytes and apoptotic cells were quantified by CAE scoring and TUNEL scoring, respectively, and were elevated by ethanol feeding. Gene expression of endoplasmic reticulum stress markers were elevated due to ethanol feeding and furthermore with PCB 126 exposure. Plasma albumin and its hepatic expression were similarly decreased by 50%, indicating hepatocellular dysfunction.

Liver functionality was compromised in this model where PCB126 altered energy metabolism to enhance ALD severity. Our future studies include elucidating mechanisms of metabolic disruption and liver injury in this multi-hit model. This study is relevant to millions of individuals who consume excessive alcohol while inevitably exposed to environmental toxicants, and this interaction is largely understudied.

Abstract # PHD 9

Abstract Title
Double-edged sword nature of N-acetyltransferase polymorphisms

Authors

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Arylamine *N*-acetyltransferases (NATs) are responsible for the metabolism and activation/detoxification of arylamines, arylhydrazines, and arylhydroxyarylamines. NATs are highly polymorphic in human populations due to SNPs that lead to numerous NAT1 and NAT2 alleles. Molecular epidemiologic studies have reported associations between the NAT genetic polymorphism and cancer risk (*e.g.,* bladder, breast, lung, prostate, and pancreatic cancers). Our study objective was to investigate the effect of the NATs genetic polymorphisms on the metabolism and genotoxicity of different carcinogens (benzidine, 3,4-dimethylaniline [3,4-DMA], β-naphthylamine [BNA] and 4,4'-methylenebis 2-chloroaniline [MOCA]). We used DNA repair deficient Chinese Hamster ovary (CHO) cells that stably express either human NAT1 alleles (*NAT1*4* and *NAT1*14B*) or human NAT2 alleles (*NAT2*4*, *NAT2*5B* and *NAT2*7B).* CHO cells were exposed to carcinogens for up to 48 h. We measured the *N*-acetylation products using HPLC and determined kinetics for the *N*-acetylation of different carcinogens. Next, we measured double stranded DNA breaks represented by γH2AX signal expression via "in cell" Western and reactive oxygen species by DCFDA assay. Lastly, we accessed mutagenicity using HPRT mutation assay. We found that acetylation of these carcinogens was modified by NAT genetic polymorphisms. *NAT1*14B* had higher *N*-acetylation rate for benzidine and showed higher level of DNA damage and HPRT mutants compared to *NAT1*4*. However, for 3,4-DMA the *NAT1*4* had higher *N*-acetylation rate, but lower mutations compared to *NAT1*14B*. For BNA and MOCA, *NAT2*4* had higher *N*-acetylation rates, DNA damage and ROS compared to NAT2*5B or NAT2*7B. Interestingly, *NAT2*7B* had higher mutation frequency than both *NAT2*4* and *NAT2*5B*. The current study presented a mammalian model with human NATs used as a tool for investigation of genotoxicity of many chemicals. Also, it provided HPLC methods for carcinogens and their acetylated products and possible biomarkers (HPRT mutants or γH2AX) that can be optimized to be used in qualitative and quantitative biomonitoring of carcinogens among the exposed populations. Finally, it suggests that human NAT genetic polymorphisms confer double-edged sword nature being protective for some carcinogens but not to others and this highlights their role as bioactivation pathway rather than detoxification. Further work would include investigations of other variant NAT alleles with different carcinogens so they can represent the broader populations.

Abstract # PHD 10

Abstract Title

TOBACCO SMOKE AUGMENTS THE PATHOGENICITY OF THE EMERGING PERIODONTAL PATHOGEN, FILIFACTOR ALOCIS.

Authors (2 lines maximum, include full first name followed by last name) M Iskander, GJ Lamont, J Tan, S Uriarte, M Pisano, DA Scott*.

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Background and Aims

Cigarette consumption increases susceptibility to multiple infectious diseases. *Filifactor alocis*, a Gram-positive anaerobe, has recently emerged as a periodontal pathogen that appears to thrive in tobacco-rich environments. Therefore, we hypothesized that identification of smoke-responsive *F. alocis* genes would provide insight into adaptive strategies and that *F. alocis* may induce alveolar bone loss in mice in a manner that was exacerbated by exposure to mainstream research cigarette smoke.

Methods

Tobacco-responsive genes were identified in *F. alocis* exposed to cigarette smoke extract (1000 ng/ml nicotine equivalents) by RNAseq, while *F. alocis* infectivity and the host response to infection was examined in a ligature-induced model of acute periodontitis in mice.

Results

F. alocis growth rates were unaffected by cigarette smoke extract exposure and only a relatively small number of genes were ascribed as being differentially regulated in manner specific to cigarette smoke conditioning. In mice exposed to mainstream smoke, relative to those exposed to ambient air, *F. alocis* infectivity was increased while differentials in mean animal mass, humoral and innate response markers, and alveolar bone loss were apparent.

Conclusions

F. alocis appears well-adapted to tobacco smoke, as implicated by a relatively low tobacco-specific transcriptional response and growth that is unaffected by smoke-exposure. Further, *F. alocis* pathogenesis is enhanced by tobacco smoke exposure, defined by increased acute alveolar bone crest to cementoenamel junction bone loss, in a manner concomitant with smoke-related alterations to *F. alocis* infectivity and to both the non-specific and adaptive arms of the immune response.

Abstract # PHD 11

Abstract Title
Novel Discovery of Copper in Modulating Neurogenesis in Adult Brain

Authors
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Adult neurogenesis occurs in brain subventricular zone (SVZ). Early studies from this group provide strong evidence for selective Cu enrichment in adult SVZ as compared to other brain regions. Our more recent data further reveal an increased neural proliferation in the SVZ, but with impaired olfaction, in experimentally Cu-deficient adult rats. However, the question as to whether Cu directly affected SVZ adult neurogenesis remained unanswered. We designed a strategy in this report to reduce SVZ Cu level by Cu chelation and to investigate the ensuing changes of SVZ neurogenesis in adult mice. An intracerebroventricular (icv) infusion technique was established to deliver Cu chelator D-Penicillamine (D-Pen), which was stored in a pump reservoir implanted under back skin, directly into the cerebrospinal fluid (CSF) by lateral ventricle cannulation. Atomic absorption spectrometry (AAS) analyses of Cu verified the efficacy of icv infusion of D-Pen, showing that the Cu levels in SVZ were reduced by 13.1% (p=0.19) and 21.4% (p<0.05), when animals received icv-infusions of D-Pen at low (0.075 μg/h) and high (0.75 μg/h) doses for 28 days, respectively, compared to saline-infused controls. Confocal analyses of the SVZ-rostral migratory stream (RMS)-olfactory bulb (OB) axis revealed that the 7-day low-dose D-Pen infusion significantly increased neural proliferation in SVZ by 28% (n=3, p<0.05) compared to controls. Using BrdU lineage tracing to quantify BrdU(+)/DCX(+) newborn neuroblasts in the RMS and OB, our data further revealed that the short-term, low-dose D-Pen infusion resulted in more newborn neuroblasts in OB than the control; however, the high-dose D-Pen infusion led to fewer newborn neuroblasts in OB but with more in the RMS (n=3, p<0.05) compared to controls. Moreover, the long-term (28-day) infusion studies revealed a significant increase of newborn neurons in OB by 37.8% (p<0.05) by low-dose D-Pen infusion but no changes by the high-dose infusion. Further investigation on critical Cu regulatory proteins indicated that both CTR1 and MT3 expressed as clusters in SVZ, and their expression was altered in response to Cu chelation. Noticeably also, CTR1 expression in the choroid plexus, a tissue nearby SVZ modulating CSF Cu homeostasis, was upregulated by long-term D-Pen infusion. In conclusion, this study provides the first-hand evidence that a reduced Cu level in SVZ by Cu chelation activated adult neurogenesis in this largest adult neurogenic region. This novel discovery has the potential to create a new avenue for understanding Cu-associated neurodegenerative diseases such as Wilson's disease, Parkinson's disease, and Alzheimer's disease.

Abstract # PHD 12

Abstract Title

Mechanisms of Benzene-Induced Endothelial Injury: Role of Heat Shock Proteins

Authors

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Benzene is a ubiquitous environmental pollutant and one of the top 20 chemicals produced in the United States. It is formed through natural processes along with human activity and is abundant in automobile exhaust and cigarette smoke. Our studies have shown that in humans, exposure to benzene is positively associated with circulating levels of endothelial microparticles, a sub-clinical marker of endothelial injury. Microparticles are often shed from activated and injured endothelial cells. Increased levels of endothelial microparticles have been associated in pathological and toxicology conditions such as diabetes and smoking. To test the plausibility that exposure to benzene is sufficient to increase endothelial microparticle levels, we exposed male C57BL/6 mice to benzene (50 ppm, 6h/day, 5days/week) via inhalation for two weeks and analyzed the microparticle levels in peripheral blood by flow cytometry. Our data show that benzene exposure increased the blood endothelial microparticle and activated endothelial microparticle levels by 2-4 fold ($P<0.05$). *In vitro* studies showed that benzene metabolite t,t,-muconaldehyde (MA, 10 micro molar) causes the apoptosis of human aortic endothelial cells (HAEC) as assessed by caspase-3 activation, and increased adhesion and transmigration of THP-1 cells. MA also robustly induced the expression of oxidative stress responsive genes superoxide dismutase 1, heme oxygenase-1, and cyclooxygenase-2, *Akr1b10*, ER-stress responsive gene *Atf3,* adhesion molecules *Icam-1* and P-selectin, and heat shock proteins *Hspa1a*, *Hspa1b*, *Hspa6*, and *Hspa* 7; and downregulated endothelial nitric oxide synthase. siRNA-mediated knockdown of *Hspa1b* augmented the cleavage of caspase-3 and caspase-7 in t,t,-muconaldehyde-treated HAEC, suggesting that downregulated endothelial nitric oxide synthase is causally involved in endothelial cell apoptosis. These data suggest that *Hspa1b* prevents benzene-induced endothelial activation/injury.

Abstract # PHD 13

Abstract Title

The Unloading of Cohesin from Chromatin a New Mechanism for Hexavalent Chromium-Induced Carcinogenesis

Authors

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Hexavalent chromium [Cr(VI)] is an environmental and occupational lung carcinogen. Inhaled Cr(VI) accumulates at bifurcation sites where it dissolves overtime leading to tumorigenesis. Although it is not completely known how it induces cancer, chromosome instability is central to its carcinogenic mechanism. Previous studies show Cr(VI)-treated cells have premature centromere spreading, a form of chromosome instability characterized by a lack of cohesion in the centromere and but not in the chromosome arms. Cohesin is a ring protein complex whose functions are to keep genomic stability, and thus its dysregulation directly leads to chromosome instability, making it a key target in our research. Cohesin is regulated by three mechanisms: localization, loading and unloading of cohesin from the chromatin. Here, we hypothesize Cr(VI) dysregulates key regulatory proteins for unloading of cohesin complex from the chromatin, separase, PDS5B, PDS5A, and WAPL**.** PDS5A/B and WAPL remove cohesin from chromosome arms during mitosis, whereas separase removes cohesin from the centromere. To test this, human lung cells were exposed to various zinc chromate concentrations for 24 and 120 h, lysed and prepared for western blot analysis and siRNA knockdowns were performed to confirm band localization. Our results show PDS5B and WAPL protein levels increase after 24 h of Cr(VI) exposure, but this effect is lost after 120 h. This trend matches previous data showing a similar result with RAD51 foci after Cr(VI) exposure that results in the inhibition of the homologous recombination repair (HR) pathway. PDS5B is also known to participate in HR repair, thus together with WAPL, they might contribute with RAD51 to the inhibition of HR. In addition, active separase, measured by cleaved levels, increased after 120 h exposure. These data together could explain Cr(VI)-induced premature centromere spreading observed after 120 h exposure, where active separase might remove cohesin from the centromeres, but lack of PDS5B and WAPL could lead to the inability to remove cohesin from the chromosome arms. PDS5A protein levels did not change. All in all, Cr(VI) dysregulated key proteins that unload cohesin from the chromatin, which can lead to chromosome instability, a key step in Cr(VI) carcinogenesis.

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Abstract # PHD 14

Abstract Title

Polychlorinated biphenyls alter hepatic m6A mRNA methylation in a mouse model of environmental liver disease

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Exposure to high fat diet (HFD) and polychlorinated biphenyls (PCBs) has been associated with liver injury in human cohorts and with nonalcoholic steatohepatitis (NASH) in mice fed a high fat diet (HFD). N(6)-methyladenosine (m6A) modification of mRNA regulates transcript fate, but the contribution of m6A modification on transcript regulation in PCB-induced steatosis and fibrosis is unknown. This study tested the hypothesis that PCB and HFD exposure alters the levels of m6A modification in transcripts that play a role in NASH *in vivo.* Male C57Bl6/J mice were fed a HFD (12 wks.) and administered a single oral dose of Aroclor1260, PCB126, or Aroclor1260 + PCB126. Genome-wide identification of m6A peaks in liver was accomplished by m6A mRNA immunoprecipitation sequencing (m6A-RIP-seq) and the mRNA transcriptome was identified by RNA-seq. Exposure of HFD-fed mice to Aroclor1260 decreased the number of m6A peaks and m6A-containing genes relative to PCB vehicle control whereas PCB126 or co-exposure to Aroclor1260+PCB126 increased m6A modification frequency. ~ 41% of genes had one m6A peak and ~49% had 2-4 m6A peaks. 117 m6A peaks were common in the four experimental groups. The Aroclor1260 + PCB126 co-exposure group showed the highest number (52) of (differentially expressed genes) DEGs with an altered number of m6A-peaks. Apob (Apolipoprotein B) was selected to validate m6A changes between PCB-specific exposures because the number of m6A peaks increased with exposure to Ar1260 (3), PCB126 (10), and Ar1260 + PCB126 (8) compared to PCB vehicle control. We selected three separate m6A-immunoprecipitated fragments that aligned to the mouse reference genome for the Apob transcript and identified all possible RRACH motifs. We designed primers for each fragment to produce an amplicon containing at least one RRACH motif. m6A-RIP-qRT-PCR confirmed enrichment of m6A-containing fragments of the Apob transcript with PCB exposure. Integrated analysis of m6A-RIP-seq and mRNA-seq identified 242 differentially expressed genes (DEGs) with increased or reduced number of m6A peaks. Comparative pathway analysis identified lipoprotein/ lipid metabolism and pro-inflammatory cytokine signaling, consistent with mechanisms of steatohepatitis in human PCB epidemiological studies. These data show that PCB exposure in HFD-fed mice alters the hepatic m6A landscape offering an additional layer of regulation of gene expression affecting a subset of gene responses in NASH.

Abstract # PHD 15

Abstract Title

Toxicological findings support the safety of a recombinant cholera toxin B subunit variant with therapeutic potential in ulcerative colitis

Authors

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Background: Epicertin (EPT) is a recombinant variant of cholera toxin B subunit that exhibits mucosal healing activity through activation of an unfolded protein response and transforming growth factor-β signaling in colon epithelial cells. We hypothesize that the unique epithelial repair activity of EPT may offer novel therapeutic strategies in diseases characterized by chronic mucosal wounds, such as ulcerative colitis. However, the pharmacokinetic and safety profiles of EPT have yet to be established, which are critical components for its development as a drug candidate.

Objective: Here, we evaluated the pharmacokinetic and toxicity profiles of intrarectally administered EPT in preclinical rodent models towards first-in-human clinical testing of an EPT enema product for ulcerative colitis treatment.

Results: A dose-escalation study was performed following a single intrarectal exposure at 1, 2 and 5 μM (61.4, 122.8 and 307 μg/animal) in male and female Sprague Dawley rats. No drug-related adverse effects were observed for clinical observations, clinical pathology, and gross necropsy even at the highest dose tested. A pharmacokinetics study was performed in male and female mice dosed a 1 or 10 μM (6.1 and 61.4 μg/animal) IV bolus EPT. Plasma samples were collected up to 24 h postdose. EPT concentrations were highest at first collection and decreased steadily until unquantifiable by 4 h. The elimination phase half-life was 0.26 to 0.3 h. When healthy (n = 36) and dextran sulfate sodium colitis mice (n = 36) were dosed 1 or 10 μM EPT intrarectally, marginal amounts of EPT were found in only 4 plasma samples scattered across groups and time points, suggesting that the drug remains at the mucosa and systemic exposure after intrarectal administration is negligible.

Conclusion: No significant toxicity associated with intrarectally administered EPT was observed in this study. The no-observed-adverse-effect-level (NOAEL) was determined to be ≥5 μM (5.2 μg drug/cm^2 of colorectal surface area), which is ≥14-times the anticipated clinical dose**.** These data support further development of EPT enema as a potential therapeutic for ulcerative colitis.

Abstract # PHD 16

Abstract Title

Acute Toxicity of 1,3-Butadiene Exposure on Human Bronchial Epithelial Cells at the Air-Liquid Interface

Authors
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Volatile organic compounds (VOCs) are ubiquitous constituents of air pollution that pose significant adverse health effects to populations worldwide. Particularly, 1,3-butadiene is a petroleum product used in the production of synthetic rubber and emitted from combustion sources such as automobile exhaust, tobacco smoke, and wood fires. Epidemiological studies have found that occupational exposure to 1,3-butadiene is associated with leukemia, and population-based studies performed at the University of Louisville Superfund Research Center suggest that 1,3-butadiene exposure is associated with pulmonary and cardiovascular injury. In addition to occupational exposures, individuals living in industrialized cities or near Superfund sites are exposed to ambient levels of 1,3-butadiene, which may have consequential long-term adverse health effects. While conventional submerged methods of *in vitro* exposures have been crucial for investigating toxicity of exogenous compounds, they lack physiological relevance to human inhalation exposures. Thus, to investigate the toxicity of 1,3-butadiene inhalation, a Vitrocell air-liquid interface (ALI) exposure system was utilized to deliver an acute sublethal dose to human bronchial epithelial cells (BEAS-2B). BEAS-2B cells were cultured on transwell inserts in a 12-well plate for ~21 days to achieve confluency and generate a pseudostratified epithelium. Apical cell media was removed prior to exposures to create air-liquid interface conditions, and the cloud generated by the ALI exposure system was allowed to deposit on the cell surface for 50 minutes. Transepithelial electrical resistance (TEER) measurements, cells, and basolateral media were collected 24 hours post-exposure to evaluate membrane integrity, cytotoxicity, and gene expression. Exposure to 1,3-butadiene significantly decreased the transepithelial electrical resistance of the cell monolayer (1.5-fold) without causing overt cell death, which suggests compromised integrity of cell junctions and increased permeability. Ingenuity Pathway Analysis of the RNA sequencing data revealed that 36 of the top 95 dysregulated protein-encoding genes were associated with the function of small cell lung carcinoma, including genes associated with canonical pathways of *FAK* and *eNOS* signaling. Further studies are required to elucidate the molecular mechanisms and full toxicological impact of 1,3-butadiene exposure on human health. Overall, ALI approaches provide an enhanced depiction of human exposures and may serve as useful tools for assessing inhalation toxicology endpoints.

Abstract # PHD 17

Abstract Title

A Mechanistic Approach to Categorizing Cr(VI) as a Gerontogen Using a Toxic Aging Coin

Authors

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The world's population is rapidly aging and in 8 years 20% of the population in developed nations will be geriatric (65+). A rapidly aging population, coupled with ubiquitous environmental pollution, creates an imminent threat to our healthcare infrastructure and necessitates greater interaction between the fields of toxicology and aging. Because of the prevalent threat of environmental pollution and the new emphasis that must be placed on aging, we aim to assess how age affects toxicology and how toxicants may act as gerontogens to induce or accelerate biological aging, using the metaphor of a toxic aging coin. The heads side of our coin represents the effects of age on toxicological outcomes while the tails side of our coin describes the gerontogenic effects of environmental pollutants. Gerontogens are described as chemicals that accelerate biological aging. Geriatric individuals exhibit elevated levels of genomic instability, which is also observed in preclinical neurodegenerative diseases. While this is well-known, no mechanism has been established to link aging and genomic instability. We propose heavy metals as a class of gerontogens. We hypothesize the genomic instability leads to cellular senescence and that these events will induce gerontogenic effects. Hexavalent chromium [Cr(VI)] is a widely used industrial chemical and a major environmental contaminant, making it a chemical of interest. Cr(VI) was chosen due to its well-described clastogenic effects and the significant lack of data concerning the neurotoxic effects of Cr(VI). At the moment, there are no federal or state regulations for Cr(VI) that consider neurotoxicity. We used M059K and M059J cells to model the effects of Cr(VI) on the hallmarks of aging, such as cellular senescence. M059J cells lack a DNA-Protein Kinase (DNA-PKcs) and are unable to repair DNA double strand breaks, whereas M059K cells have functional DNA-PKcs and are proficient in break repair. We show M059J cells are more susceptible to Cr(VI) cytotoxicity than M059K and escape Cr(VI)-induced growth arrest. Our results indicate DNA damage repair signaling must be intact for growth arrest and cellular senescence. Further studies will investigate a mechanism relating DNA repair pathway dysfunction to cellular senescence. This work is supported by R21ES033327 (JPWJr), R35ES032876 (JPWSr), and P30ES030283.

Abstract # PHD 18

Abstract Title

Hepatic metabolism of heterocyclic amines contributes to induction of glucose production and gluconeogenic gene expression in hepatocytes

Authors

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Heterocyclic amines (HCAs) are mutagens generated when cooking meat at high temperatures or until well-done. Their major metabolic pathway involves hepatic *N*-hydroxylation via CYP1A2 followed by *O*-acetylation via arylamine N-acetyltransferase 2 (NAT2). *NAT2* expresses a well-defined genetic polymorphism in humans that results in clinical differences in metabolizing its substrates, including HCAs. Recent epidemiological studies reported that dietary HCA exposure may be associated with higher incidence of insulin resistance and type II diabetes. However, effects of HCAs on insulin sensitivity or glucose homeostasis have not yet been reported. We hypothesized that 1) HCAs directly alter glucose homeostasis (and insulin sensitivity) in human hepatocytes, and that 2) hepatic metabolism of HCAs contributes to this effect. Cryopreserved human hepatocytes or HepG2 (hepatocellular carcinoma) cells were treated with HCAs (2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline [MeIQ], 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline [MeIQx], or 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine [PhIP]). Glucose production was measured via glucose oxidase assay, and gluconeogenic gene (*G6PC* and *PCK1*) expression was analyzed via qRT-PCR. To investigate the role of HCA metabolism, we co-treated HepG2 cells with MeIQx and 3-methylcholanthrene (3-MC), a cytochrome P450 inducer, and measured gluconeogenic gene expression. We also measured and compared glucose production in HCA-treated cryopreserved human hepatocytes with distinct NAT2 acetylator phenotypes (i.e., rapid, intermediate, and slow). Differences in relative gene expression and glucose production between the treatment groups vs. control groups were tested for significance by one-way ANOVA followed by Dunnett's Comparison Test. Dose-response linear trend was tested for significance by the post test for linear trend using a linear regression model. HCAs significantly induced gluconeogenic gene expression in hepatocytes and HepG2 cells. This effect was more pronounced in HepG2 cells following co-treatment with 3-MC. Additionally, HCAs increased glucose production in hepatocytes in a significant dose-dependent manner. This effect was augmented in hepatocytes with rapid NAT2 acetylator genotype, compared to intermediate and slow acetylator genotype. These results suggest that consumption of HCAs contributes to the development of hyperglycemia and insulin resistance. In addition, the metabolic effect of HCAs is augmented by hepatic and NAT2-mediated metabolism.

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Abstract # PHD 19

Abstract Title

Alcohol Activates Dgat2 through α4 Nicotinic Cholinergic Receptors in Hepatocyte Models

Authors

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Steatosis, or fatty liver, is one the earliest manifestations of excessive alcohol consumption. It is characterized by triacylglyceride accumulation within hepatocytes. Upregulation of 1,2-diacylglycerol acyltransferase (Dgat2), the rate-limiting enzyme in triglyceride synthesis, contributes to steatosis in response to alcohol. Recently, we showed that α4 nicotinic acetylcholine receptor (α4 nAChR, Chrna4) knockout mice did not develop fatty livers upon chronic exposure to alcohol, suggesting that these receptors are involved in the regulation of Dgat2 expression. The purpose of the present study was to test the hypothesis that alcohol induces the expression of Dgat2 by acting on α4 nAChRs expressed in hepatocytes. Alcohol's effects were investigated in cultured AML12 hepatocytes exposed to 100 mM alcohol for up to 3 days, primary mouse hepatocytes exposed to 60 mM alcohol for up to two days, and in the livers of wild type and α4 nAChR knockout mice fed the Lieber-DeCarli liquid alcohol diet for 6 weeks. Levels of Dgat2 and Chrna4 were measured by quantitative RT-PCR and/or western blotting. Data was analyzed using GraphPad Prism by One- or Two-Way ANOVA followed by Tukey's post-hoc test for multiple comparisons with significance set at $p < 0.05$. In AML12 hepatocytes, primary mouse hepatocytes, and wild type mouse livers, alcohol increased the expression of Dgat2 and Chrna4, the gene encoding the α4 subunit of nAChRs. In the livers of α4 nAChR knockout mice, there was no detectable expression of Chrna4 with or without exposure to alcohol. Importantly, Dgat2 levels were unaffected by alcohol in the knockout mice. Taken together, these data support a role for α4 nAChRs expressed by hepatocytes in mediating the steatotic effects of alcohol on the liver. There are currently no FDA approved therapies for any stage of alcohol-associated fatty liver disease. The present studies identify α4 nAChRs as novel targets useful in the treatment and management of alcohol induced steatosis.

Abstract # PHD 20

Abstract Title

Particulate Hexavalent Chromium Exposure Induces RAD51D Loss Leading to Impaired function with Each Successive Generation in Human Lung Cells

Authors

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Lung cancer is the leading cause of cancer deaths, and it is frequently attributed to smoking as its cause. However, smoking cannot account for substantial amounts of the disease and understanding how other agents cause lung cancer is a critical need. Environmental metal exposure also causes a significant amount of lung cancer. Metals cause lung cancer by inducing chromosome instability (CIN), but mechanism for how they induce CIN are poorly understood. We focused on hexavalent chromium [Cr(VI)] as a representative metal as it is a known human lung carcinogen that induces DNA double strand breaks (DSBs) while simultaneously inhibiting the repair of those breaks resulting in CIN. The homologous recombination (HR) repair pathway prevents CIN by repairing DSBs. Cr(VI) targets RAD51, a key protein within the HR pathway, and prevents its loading onto a filament. RAD51 paralogs orchestrate RAD51 function consisting of two complexes: BCDX2 and CX3. It is unknown if Cr(VI) impacts these complexes and if this effect is transient or persistent at a cellular level. We hypothesize that **Cr(VI) inhibits HR repair by targeting the complexes responsible for RAD51 filament loading and that impairment is persistent in human lung cells.** We measured effects on RAD51D and XRCC3 as representatives of the BCDX2 and CX3 complex. Effects were measured using immunofluorescent foci formation for DNA repair, western blot for protein levels, and QRTPCR for mRNA levels. We found Cr(VI) inhibited RAD51D foci formation, reduced protein levels, and gene expression after acute and prolonged exposures. We also found the decrease in RAD51D foci formation was much more pronounced in the subsequent treated generations indicating Cr(VI)-induced foci inhibition is persistent. In contrast, we found Cr(VI) had less impact on XRCC3 foci formation suggesting the CX3 complex is less affected. This data suggests RAD51D as a part of the BCDX2 complex may be key in Cr(VI)-reduced RAD51 function and HR repair inhibition. Completion of this study will identify key mechanisms for how hexavalent chromium causes CIN and lead to new ways to avoid lung cancer in general. Supported by NIEHS grants R01ES016893 and R35 ES032876 (J.P.W.) and T32-ES011564 (A.W. and J.P.W.).

Postdoctoral Fellow Abstracts

Abstract # PD 1

Abstract Title

Electronic Cigarette-derived Aerosols, Endothelial Dysfunction, and Platelet Activation in Mice: Role of Acrolein and Formaldehyde

Authors

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Introduction: Electronic cigarettes (e-cigs) threaten to reverse the decline in rates of nicotine dependence and smoking. While some believe that e-cigarettes are less harmful than combustible cigarettes, e-cig use is associated with respiratory symptoms (cough, wheeze) and endothelial dysfunction (ED) indicating a potential to increase the risk of cardiovascular disease (CVD) with chronic use. ED is *sine qua non* of multiple CVD, yet the mechanism of e-cig-induced ED is unknown.

Hypothesis: E-cigs generate levels of irritants that activate the Transient Receptor Potential Ankyrin-1 (TRPA1) to trigger nocifensive reflexes and induce ED in mice.

Methods: Wild type (WT) and TRPA1-null C57BL/6J male and female mice were exposed to: filtered air (control), aerosols of e-cig (JUUL) liquid, e-cig solvents without nicotine (propylene glycol:glycerin, PG:G), or irritant gases alone (acrolein, formaldehyde). Urinary levels of nicotine and aldehyde metabolites were measured by UPLC-MS/MS. During acute exposures, respiratory irritant responses were monitored by radiotelemetry. Following short-term exposure (4d), aortic endothelial function, blood/plasma markers of organ toxicity, and platelet activation were measured.

Results: Exposure of WT mice to e-cig aerosols increased urine levels of the acrolein metabolite (3HPMA) but not of formaldehyde (formate), equivalent to that of exposures to 1 ppm acrolein alone. In WT mice, exposure to either PG:G (30:70), formaldehyde (5 ppm), or acrolein (1 ppm) induced respiratory braking. In female WT mice (n=5-18), exposure to PG:G (-61.8±4.2 % ACh relaxation) and formaldehyde (−56.3 ± 4.5 %) but not acrolein (-80.0±3.2 %) induced ED vs control group (-77.8±1.5 %). In female TRPA1-null mice (n=5-14), neither PG:G (-77.6±3.6 %), formaldehyde (-85.9±7.8 %) nor acrolein (-86.6±2.8 %) exposure induced ED vs control (-81.3±5.4 %). Acrolein exposure enhanced aortic sensitivity to an NO donor similarly in female WT and TRPA1-null mice.

Conclusions: Formaldehyde and acrolein generated from the PG:G in e-cigs provoked pulmonary irritant reflexes, and formaldehyde exposure alone induced ED in female mice. Irritant aldehydes in e-cig-derived aerosols activate the TRPA1 receptor to promote ED that could increase CVD risk.

Abstract # PD 2

Abstract Title

Vitamin D Metabolite Enhances Corticosteroid Sensitivity and Modulates Inflammatory Pathways in Severe Allergic Airway Inflammation

Authors

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Introduction: Severe asthma involves complex inflammatory pathways involving innate and adaptive immune cells that secrete effector cytokines to promote asthma pathogenesis. Airway inflammation and remodeling persists despite treatment with corticosteroids, increasing asthma symptoms and exacerbations. A deficiency in vitamin D metabolism is associated with development of asthma and may also affect corticosteroid sensitivity. We evaluated the effects of Vitamin D_3 and its metabolite ,1,25-dihydroxy vitamin D_3 (calcitriol), on corticosteroid sensitivity in a mouse model of severe allergic airway inflammation.

Methods and Results: To induce allergic airway inflammation, newborn mice were challenged with PBS or mixed allergen (MA; *Aspergillus fumigatus*, house dust mite, *Alternaria alternata*, and ovalbumin) and c-di-GMP, for 7 weeks. Mice were also administered vehicle, 1 mg/kg fluticasone propionate (FP), and/or 50 ng/g of Vitamin D_3 or calcitriol. Lung inflammation and airway hyperresponsiveness were analyzed. Lung tissue was collected for bulk RNA-sequencing for differential and pathway analyses. Compared to PBS mice, c-di-GMP + MA increased immune cell infiltration and airway hyperresponsiveness. Treatment with Vitamin D_3 and calcitriol along with FP reduced total immune cell infiltration. However, treatment with calcitriol reduced airway hyperresponsiveness whereas it remained enhanced with Vitamin D_3 treatment. Whole lung RNA-seq analyses on lung tissue revealed that 1 mg/kg FP and calcitriol significantly reduced more than 1100 genes, which are associated with immune cell infiltration and pro-inflammatory pathways.

Conclusions: These data suggest that a vitamin D_3 metabolite, calcitriol, can alleviate severe airway inflammation by improving corticosteroid sensitivity. Enhancement of corticosteroid sensitivity by calcitriol may provide a therapeutic approach to improve corticosteroid sensitivity in patients with severe asthma.

Abstract # PD 3

Abstract Title

Translating Particulate Hexavalent Chromium-Induced Genotoxic and DNA Repair Impacts from Human Lung Cells to *In Vivo* Lung Tissue

Authors

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Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen, but how Cr(VI) induces lung cancer is still uncertain. Chromosome instability (CIN) , a hallmark of lung cancer, is considered a major driving factor in Cr(VI)-induced lung cancer. Studies in cultured human lung cells show particulate Cr(VI) induces DNA double strand breaks in the late S and G2 phases of the cell cycle when homologous recombination (HR) repair is the main repair pathway for fixing the breaks. At the same time, our previous data show Cr(VI) suppresses HR repair by targeting RAD51 and the combination of breaks with failed repair results in CIN. However, these observations have only been reported in cell culture studies. We translated these outcomes to rats, as this species develops Cr(VI)-induced lung tumors. 12-week-old male and female Wistar rats were exposed to either zinc chromate particles in a saline solution or saline alone by oropharyngeal aspiration. There were two timepoints: single dose for 24 hours and a single dose repeated once weekly for 90 days. Cr was found in every rat lung lobe with more Cr found in the right lung. We found both DNA double strand breaks and HR repair increased in a concentration-dependent in rat lungs after 24-hour Cr(VI) exposure. However, after 90 days of exposure, we found DNA double strand breaks increased, but HR repair decreased. Notably, these effects were distinct in bronchioles and more muted in alveoli. We also considered these outcomes in Cr(VI)-associated human lung tumors, and found DNA double strand breaks increased and RAD51 levels decreased in lung tumor tissue compared to adjacent normal lung tissue Thus, we successfully translated Cr(VI)-induced DNA double strand break and HR repair inhibition from cells to experimental animals, normal human lung tissue, and Cr(VI)-associated human lung tumors. Our findings established a mechanism for Cr(VI)-induced DNA double strand break and HR repair inhibition in Cr(VI) carcinogenesis. This work was supported by the National Institute of Environmental Health Sciences [R35ES032876 and ES016893 to JPW] and the University of Louisville School of Medicine Basic Grants Program (SSW).

Abstract # PD 4

Abstract Title
Chronic Arsenic Exposure Reduces ATM Pathway Activation in Human Keratinocytes

Authors
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Background: Inorganic arsenic (iAs) is a class I human carcinogen. Approximately 225 million people, including >2 million in the U.S., are exposed to high iAs concentrations (>10 μg/L) by drinking contaminated water. Skin is a major target organ of iAs, and chronic iAs exposure results in hallmark skin lesions including iAs-induced skin cancer. iAs is also well-recognized as a clastogen; iAs exposure is associated with increased DNA double-strand break (DSB) accumulation in human populations and *in vitro*. Structural chromosomal instability (CIN), characterized by DSBs, is a hallmark of cancer. Since iAs does not directly interact with DNA, it is likely that other mechanisms, such as inhibition of DSB repair and signaling, may be responsible for iAs-induced CIN.

Hypothesis: Chronic iAs exposure results in CIN by suppression of ATM pathway activation in human keratinocytes.

Methods: Passage-matched, quadruplicate cultures of human keratinocytes (HaCaT or Ker-CT cells) were chronically exposed to media containing 0 or 100 nM iAs. Protein lysates collected at 7 (HaCaT) or 8 (Ker-CT) weeks were examined by immunoblotting for DNA damage response (DDR) proteins (*i.e.* MRE11-RAD50-NBN (MRN) complex, ATM and downstream signaling proteins, ATR) and phosphatases which resolve DDR signaling. Additionally, neutral COMET assays were performed to assess DSBs. Dose-response and time course experiments with neocarzinostatin (NCS) were used to analyze ATM activation kinetics in Ker-CT cells either unexposed or chronically exposed to iAs. Appropriate statistical tests (unpaired two-tailed t-test, One or Two-Way ANOVA), depending on the nature of the data, were performed with GraphPad Prism software; p-value <0.05 was considered significant.

Results: Significantly increased DSBs and decreased phosphorylated ATM and CHEK2 occurred concomitantly in both human keratinocyte cell lines chronically exposed to iAs indicating that chronic iAs exposure significantly reduced ATM pathway activation in both cell lines.

Conclusions: Chronic iAs exposure impaired ATM pathway signaling, possibly through reduced initial DSB detection and activation of the MRN complex. These results suggest that homologous recombination (HR, error-free) repair is likely repressed in iAs exposed cells and preferential use of non-homologous end joining (NHEJ, error-prone) repair could contribute to iAs-induced CIN and carcinogenesis.

Abstract # PD 5

Abstract Title

Effect of *N*-Acetyltransferase Polymorphism on Metabolism and Genotoxicity of 4,4'-Oxydianiline (ODA)

Authors

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Introduction

The use of hookah smoking is increasingly popular. 4,4'-Oxydianiline (ODA), is a main component in the hookah smoke. The metabolism and the subsequent toxicity of ODA to hepatocytes and immortalized human lung cells remains unknown. Given, that ODA is an aromatic amine, its toxicity may be dependent on metabolism by arylamine *N*-acetyltransferase 2 (NAT2). Further, it is possible that NAT2 genetic polymorphism affects the toxicity risk. Arylamine *N*-acetyltransferase 2 capacity is subject to a genetic polymorphism in human populations.

Hypothesis

We hypothesize that 4,4'-Oxydianiline is acetylated by NAT2 and its genetic polymorphism modifies ODA genotoxicity and oxidative stress.

Methods

We measured the *N*-acetylation of ODA by NAT1 and NAT2 using HPLC. We used the repair deficient (UV5) Chinese hamster ovary cells (CHO) expressing CYP1A2 with either the *NAT2*4* or *NAT2*5B* allele variants and cryopreserved human hepatocytes expressing rapid, intermediate, or slow NAT2 genotypes. We measured the induction of DNA double-strand breaks (γH2Ax protein expression) and oxidative stress (DCFDA assay). To further demonstrate the genotoxicity and metabolism in an inhalation cell culture model, *N*-acetylation and genotoxicity of ODA were investigated in immortalized human lung epithelial cells (BEP2D) expressing NAT2. Statistical analyses included: One-Way ANOVA followed by Dunnett's Multiple Comparison, and simple linear regression analysis.

Results

We first compared *N*-acetylation by human *N*-acetyltransferase 1 and NAT2. We found that *N*-acetylation of ODA is carried out exclusively by NAT2. In CHO cells, expressing *CYP1A2* and either *NAT2*4* (reference allele) or *NAT2*5B* (common variant allele), the induction of γH2Ax was higher in the *NAT2*4* vs *NAT2*5B* expressing CHO cells ($p<0.05$). Induction of oxidative stress was also higher in *NAT2*4* vs *NAT2*5B* ($p<0.05$). Similar results were observed in the cryopreserved human hepatocytes, showing a dose-dependent and genotype dependent response in all the endpoints tested, with indices of toxicity higher in the rapid, vs the intermediate and the slow acetylators ($p< 0.01$). Additionally, ODA was *N*-acetylated in BEP2D cells and caused genotoxicity as measured by (γH2Ax) in a dose dependent manner ($p<0.001$).

Conclusions

These results provide evidence that exposure to ODA results in NAT2 genotype dependent genotoxicity and oxidative damage in both human hepatocytes and lung cells.

Grant Support

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Faculty and Staff Abstracts

Abstract # FS 1

Abstract Title
Zinc supplementation prevents mitotic accumulation in human keratinocyte cell lines upon environmentally relevant arsenic exposure

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Introduction: Chronic exposure to inorganic arsenic (iAs) is a global health issue leading to multi-organ diseases, often characterized by disrupted cell cycle progression. Skin cancer is a common outcome. Exposure to supratherapeutic concentrations of iAs causes cellular accumulation in G2 or M phase of the cell cycle in multiple cell lines by stabilizing cyclin B1and securin. It is not clear if iAs exposure at levels corresponding to those in serum of chronically exposed populations (~100 nM) has any effect on cell cycle distribution.

Hypothesis: Environmentally relevant iAs exposure will lead to mitotic accumulation of human keratinocytes by stabilizing ubiquitination substrates of RING finger E3 ubiquitin ligase ANAPC11.

Methods: For dose-response experiments, human keratinocyte cell lines (HaCaT and Ker-CT) were exposed to iAs (0-5 μM; 24 h). For zinc supplementation experiments, HaCaT and Ker-CT cell lines were simultaneously treated with iAs (0 or 0.1 μM; 24 h) and zinc (0 or 1 μM; 24 h). RNA and whole cell lysates were isolated for each experiment. Cell cycle distribution was measured using flow cytometry. mRNA and protein levels of ANAPC11 substrates cyclin B1 and securin were measured by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and immunoblotting respectively. Data were analyzed by One-Way ANOVA with Dunnett's post-hoc test (dose-response) or Two-Way ANOVA with Tukey's post-hoc test (zinc supplementation). For all tests, $p<0.05$ was considered significant.

Results: iAs exposure starting at 0.1 μM led to mitotic accumulation of cells in both cell lines, along with the stabilization of ANAPC11 ubiquitination targets cyclin B1 and securin, without affecting their steady state mRNA levels. Moreover, zinc supplementation prevented iAs-induced mitotic accumulation and stabilization of cyclin B1 and securin without affecting their mRNA levels.

Conclusions: Environmentally relevant iAs exposure leads to mitotic accumulation possibly by displacing zinc from the RING finger of cell cycle regulating E3 ubiquitin ligase ANAPC11. iAs-induced cell cycle disruption could underpin the molecular pathogenesis of multiple diseases associated with chronic iAs exposure. Physiologically relevant zinc supplementation prevents all the cell cycle dysregulatory effects of iAs exposure and could be an inexpensive way to combat effects of chronic iAs exposure.

Grant Support: Supported by NIH grants P30ES030283, R01ES027778 and P20GM135004.

Abstract # FS 2

Abstract Title

Attenuation of 1,2,3-trichlorobenzene nephrotoxicity in isolated kidney cells from Fischer 344 rats

Authors

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Chlorobenzenes are commonly used in the manufacture of a wide variety of commercial products and are present in soil and wastewater as environmental contaminants. Chlorobenzene toxicity includes nephrotoxicity, with in vivo nephrotoxicity associated with α2u-globulin in male rats. Our laboratory recently demonstrated that trichlorobenzenes (TCBs) are directly toxic to the kidney, with 1,2,3-TCB being the most potent nephrotoxicant among the three TCB isomers. The purpose of this study was to determine if bioactivation or free radical mechanisms contributed to 1,2,3-TCB nephrotoxicity by pretreating isolated kidney cells (IKCs) with metabolism inhibitors or antioxidants. IKC (~4 million cells/ml; 3 ml) were incubated with shaking at 37ºC under a 95% oxygen/5% carbon dioxide atmosphere with 1,2,3-TCB (1.0 mM) or vehicle (dimethyl sulfoxide) for 60 min with or without a pretreatment. General cytotoxicity was determined by determining trypan blue exclusion by IKC, measuring changes in lactate dehydrogenase (LDH) release, determining 4-hydroxynonenal (4-HNE) protein adduction, and quantitating ATP levels. 1,2,3-TCB cytotoxicity was reduced by all antioxidants tested (ascorbate, glutathione, alpha-tocopherol) and general cytochrome P450 (CYP) inhibitors (metyrapone and piperonyl butoxide), but not by peroxidase inhibitors (mercaptosuccinate, indomethacin). Several selective CYP inhibitors (isoniazid, omeprazole, sulfaphenazole, oleandomycin, thiotepa) were used as pretreatments, and all provided some protection against 1,2,3-TCB cytotoxicity. Protective pretreatments also generally attenuated 1,2,3-TCB-induced reductions in ATP levels. These results suggest that CYP-mediated biotransformation and free radicals play a role in 1,2,3-TCB nephrotoxicity in vitro, but no evidence was obtained that oxidative stress is a causal mechanism in 1,2,3-TCB nephrotoxicity. Supported in part by NIH grant P20GM103434.