NIEHS/SRP Workshop: Health Effects and Mitigation of Arsenic: Current Research Efforts and Future Directions

Poster Abstracts

Day 1: Dr. Michelle Heacock (Chair)
Judges for Day 1: Dr. Cynthia Rider; Dr. Humphrey Yao; Dr. Michelle Hooth; Dr. Sue Fenton; Dr. Janice Allen; Dr. Mike Humble

Day 2: Dr. Erik Tokar (Chair)
Judges for Day 2: Dr. Rick Woychik; Dr. Suryama Waidyanatha; Dr. Alex Merrick; Dr. Dan Morgan; Dr. Christie Sayes; Dr. Michael Hughes; Dr. Caroline Dilworth
Day 1
**Competition Entries – Day 1**

**Poster Board #1**

Fenna C.M. Sillé, Daniel Medina-Cleghorn, Martyn T. Smith, Craig M. Steinmaus, Allan H. Smith, Daniel K. Nomura

Affiliation: Superfund Research Program – UC Berkeley

Arsenic and innate immunity: macrophage function upon arsenic exposure

In a unique study area in Chile, our research group has reported that early-life arsenic exposure is associated with the greatest increase in mortality from lung and bladder cancers, myocardial infarction, pulmonary tuberculosis (TB) bronchiectasis, and other COPD ever associated with early-life environmental exposure. These risks remain high even >30 years after arsenic exposure has ceased, but the mechanisms for this prolonged effect remain unknown. We hypothesize that arsenic ingestion permanently impacts immune development and increases the risks of various immune-related diseases later in life. Here we focus on macrophages, innate immune cells known to influence tumor progression and TB pathogenesis. We performed multiplex cytokine/chemokine profiling analysis on supernatant from in vitro monomethylarsonous acid (MMA3)-treated mouse bone-marrow-derived macrophages (BMDM). Our results revealed significant downregulation of various pro-inflammatory cytokines and chemokines involved in the NOD-Like receptor and Toll-Like-receptor pathways, both critical in the innate immunity against TB. Lipid metabolomics experiments on these same BMDMs showed that arsenic treatment led to elevations in several pro-inflammatory and tumor-promoting signaling lipids known to play a role in tumor progression as well as the immunopathogenesis of TB. Follow-up studies have been planned to understand how arsenic-induced immunogenic and metabolic alterations in macrophages influence TB and tumor cell pathogenicity in vitro and in vivo.
Poster Board #3


Affiliation: Duke University

Investigating genetic susceptibility to arsenite induced mitochondrial toxicity

Genetic variation within the human populous causes a range of sensitivities to various toxicants, and increasing numbers of environmental toxicants have been identified as targeting the mitochondria. We hypothesized that these mitotoxicants pose an increased risk to individuals suffering from mitochondrial disease. To test that hypothesis, we exposed larval wild-type C. elegans and a genetic variant carrying a deletion in the fzo-1 gene to several mitotoxicants. fzo-1 and its human orthologs MFN1 and MFN2 play a major role in mitochondrial dynamics, and mutations in MFN2 cause Charcot-Marie-Tooth neuropathy type 2A in humans. Previously, we demonstrated that larval fzo-1 nematodes are extremely sensitive to mtDNA damage, and removal of damage is fzo-1-dependent. Since larval development is dependent on mitochondrial function, initial mitotoxicant screens tested for exacerbation of growth inhibition in fzo-1. Those screens demonstrated that chronic exposure to rotenone(250nM), paraquat(150µM), and arsenite(400µM) delayed the growth of fzo-1 mutants, compared to wild-type nematodes. To further investigate the mitochondrial effects of arsenite in wild-type and mitofusin-deficient backgrounds, we are measuring ATP levels and basal and maximal respiratory capacities of nematodes after arsenite exposure. Preliminary results indicate that basal and maximal oxygen consumption increase in a nonmonotonic fashion in response to arsenite, resulting in a reduced spare respiratory capacity in wild-type and fzo-1 mutant backgrounds. Strain-specific differences in the response of oxygen consumption to arsenite have not been detected. Arsenite also resulted in nonmonotonically altered ATP levels.
Poster Board #5

Brandilyn A. Peters, Megan N. Hall, Xinhua Liu, Vesna Ilijevski, Vesna Slavkovich, Abu Siddique, Shafiul Alam, Joseph H. Graziano, Mary V. Gamble

Affiliation: Columbia University

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Poster Board #7

Katherine Moon Johns Hopkins Bloomberg School of Public Health, USA
Eliseo Guallar Johns Hopkins Bloomberg School of Public Health, USA & Centro Nacional de Investigaciones Cardiovasculares (CNIC), Spain
Jason Umans MedStar Health Research Institute, Georgetown University, and Weill Medical College of Cornell Medical Center, USA
Richard Devereux Weill Medical College of Cornell Medical Center, USA
Lyle Best Missouri Breaks Industries Research, Inc., USA
Kevin Francesconi Karl-Franzens University, Austria
Walter Goessler Karl-Franzens University, Austria
Jonathan Pollak Johns Hopkins Bloomberg School of Public Health, USA
Ellen Silbergeld Johns Hopkins Bloomberg School of Public Health, USA
Barbara Howard MedStar Health Research Institute, Georgetown University, and Weill Medical College of Cornell Medical Center, USA
Ana Navas-Acien Johns Hopkins Bloomberg School of Public Health, USA

Affiliation: Johns Hopkins Bloomberg School of Public Health

Inorganic arsenic exposure in water and food is a global public health problem. Chronic exposure to high levels of arsenic is consistently associated with increased risk of cardiovascular disease, whereas prospective data on low to moderate chronic arsenic exposure (<100 µg/L in drinking water) are lacking. We aimed to examine the prospective association between chronic low to moderate arsenic exposure and cardiovascular disease. We evaluated the association between chronic arsenic exposure and incident cardiovascular disease among 3,575 men and women aged 45-74 years who participated in the Strong Heart Study in 1989-1991 and were followed through 2008. We used the sum of inorganic and methylated arsenic species in urine at baseline as a biomarker of arsenic exposure, divided by creatinine to account for urine dilution. 1,184 participants developed fatal and non-fatal cardiovascular disease. 439 participants developed fatal cardiovascular disease. Comparing the highest to lowest quartile of arsenic concentrations (>15.7 vs. <5.8 µg/g creatinine), the hazard ratios (95% confidence interval) for cardiovascular disease, coronary heart disease, and stroke mortality after adjustment for socio-demographic factors, smoking, body mass index, and lipids were 1.65 (1.20, 2.27), 1.71 (1.19, 2.44) and 3.03 (1.08, 8.50), respectively. Corresponding hazard ratios for incident cardiovascular disease, coronary heart disease, and stroke were 1.32 (1.09, 1.59), 1.30 (1.04, 1.62), and 1.47 (0.97, 2.21), respectively. Low to moderate chronic arsenic exposure, as measured in urine, was prospectively associated with cardiovascular disease incidence and mortality. These findings support arsenic at low to moderate concentrations as an independent cardiovascular disease risk factor.
Introduction: Few studies have evaluated associations between low-moderate arsenic levels and incident chronic kidney disease (CKD). Our objective is to evaluate cross-sectional and prospective associations between inorganic arsenic exposure and CKD in American Indian adults who participated in the Strong Heart Study (SHS).

Study Population: 3,851 adults aged 45 – 74 years in 1989-1991 with urine arsenic and serum creatinine measures. Prospective analyses were restricted to 3119 participants without CKD at baseline and with at least one follow-up visit between 1989 and 1999.

Measurements: Urine arsenic species were measured using HPLC-ICPMS. Plasma creatinine was measured by Jaffe method. eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) equation. CKD was defined as eGFR <60 mL/min/1.73 m², kidney transplant or dialysis.

Results: The prevalence of CKD at baseline was 10.3%. The multivariable-adjusted OR of prevalent CKD comparing the 75th to the 25th percentile for the sum of inorganic and methylated arsenic was 0.68 (0.56, 0.82) after adjustment for fasting glucose. The incidence of CKD over the study period was 16.1%. The multivariable-adjusted hazard ratio for incident CKD comparing the 75th to the 25th percentile of urine arsenic concentrations was 1.16 (1.00, 1.35) before and 1.08 (0.92, 1.26) after adjustment for fasting glucose.

Conclusion: Increasing urine arsenic concentrations were inversely associated with prevalent CKD. Baseline urine arsenic levels were positively associated with incident CKD, but not after adjustment for fasting glucose. Longitudinal studies with repeated measures are needed to further characterize the association between arsenic exposure and kidney disease.
Objective: To evaluate the prospective association between low-moderate arsenic exposure and all-cause and site-specific cancer mortality in American Indians from Arizona, Oklahoma and North and South Dakota who participated in the Strong Heart Study in 1989-91. Methods: Prospective cohort study of 3,932 American Indians 45 to 74 years of age followed through 2008. Baseline urine arsenic species (inorganic arsenic, monomethylarsonate (MMA), dimethylarsinate (DMA) and arsenobetatine plus other cations) were measured using anion exchange high performance liquid chromatography with inductively coupled plasma mass spectrometry. Exposure to inorganic arsenic was estimated as the sum of inorganic and methylated arsenic species in urine. Cancer events were assessed by annual mortality surveillance reviews and recorded according to the International Classification of Diseases, 9th Revision. Results: Median (interquartile range) urine concentration for the sum of inorganic and methylated arsenic species was 9.7 (5.8-15.6) µg/g creatinine. The adjusted Hazard Ratios (95CI) comparing the 80th vs 20th percentiles of arsenic were 1.14 (0.92-1.41) for overall cancer, 1.56 (1.02-2.39) for lung cancer, 1.34 (0.66, 2.72) for liver cancer, 3.30 (1.28-8.48) for prostate cancer and 0.44 (0.14, 1.14) for kidney cancer. The corresponding hazard ratios were 2.46 (1.09-5.58) for pancreatic cancer and 0.46 (0.22-0.96) for lymphatic and hematopoietic cancers. No association was observed for colorectal, gastro-esophageal or breast cancers. Conclusions: Our findings provide evidence of arsenic carcinogenicity at low-moderate arsenic levels and support a linear dose-response relationship with lung, prostate and pancreatic cancers with no evidence of a threshold.
Exposure to inorganic arsenic (iAs) from drinking water is a global public health problem yet much remains unknown about the extent of exposure in susceptible populations. Our objectives were to establish the Biomarkers of Exposure to ARsenic (BEAR) prospective pregnancy cohort in Gómez Palacio, Mexico, in order to better understand the effects of iAs exposure on pregnant women and their newborn children. Two hundred pregnant women were recruited for this study. Concentrations of iAs in drinking water (DW-iAs) and maternal urinary concentrations of iAs and its metabolites were determined. Birth outcomes were analyzed for their relationship to DW-iAs, the sum of urinary concentrations of iAs (U-tAs), and its monomethylated and dimethylated metabolites (MMAs and DMAs, respectively) and concentrations and proportions of individual urinary arsenicals. DW-iAs for the study subjects ranged from <0.5 to 236 μg As/L. More than half of the women (53%) had DW-iAs that exceeded the World Health Organizations recommended guideline of 10 μg As/L. DW-iAs was significantly associated with U-tAs. While DW-iAs and the sum of urinary arsenicals (U-tAs) were not associated with any birth outcomes, both maternal urinary concentrations and proportions of MMAs were associated with a decrease in newborn birth weight. Biomonitoring results demonstrate that pregnant women in Gómez Palacio are exposed to potentially harmful levels of DW-iAs. The data support a relationship between iAs metabolism in pregnant women and adverse birth outcomes. The results underscore the risks associated with iAs exposure in vulnerable populations.
Poster Board #15

Darcy Weidemann, Ana Navas-Acien, Chin-Chi Kuo, Virginia Weaver, Jeffrey Fadrowski

Affiliation: Johns Hopkins Children’s Center

Objective: To examine the association of urinary arsenic exposure and estimated glomerular filtration rate (eGFR) in a representative population of US adolescents.

Methods: Cross-sectional study in 1,379 participants 12-17 years in the 2003-2010 National Health and Nutrition Examination Survey (NHANES). Using linear regression adjusted for kidney disease risk factors, urinary dilution, and markers of seafood intake (arsenobetaine), we examined the association of urinary total arsenic (As) and dimethylarsonate (DMA) modeled as log-transformed continuous variables and quartiles with eGFR (ml/min/1.73 m²).

Results: The mean age was 14.5 years. Median total urinary As and DMA (IQR, interquartile range) was 6.7 μg/L (IQR 3.8 – 12.1) and 3.6 μg/L (IQR 2.1 – 5.5), respectively. Log-transformed total As and DMA were positively associated with GFR, with an increase of GFR by 4.5 mL/min/1.73 m² (95% confidence interval [CI] 1.9-7.1) and 3.5 mL/min/1.73 m² (CI 0.77-6.13), respectively. Quartile analysis showed, as compared to the lowest quartile, higher quartiles of As and DMA were associated with higher eGFRs. Conclusions: Higher total urinary As and DMA levels were associated with higher eGFR. This relationship could be explained by glomerular hyperfiltration induced by As. Alternatively, among this population of adolescents with eGFR in the normal range, higher eGFRs may be associated with greater As excretion, with potential implications for exposure assessment using urinary biomarkers. Prospective studies are needed to examine the relationship between eGFR and baseline As levels, and the change in urinary As levels with changes in eGFR.
No large-scale studies have evaluated whether the cardiovascular effects of arsenic exposure could be modified by genetic factors. We conducted 1) a case-cohort study of 447 incident fatal and nonfatal cases of cardiovascular disease (CVD), including 238 cases of coronary heart disease (CHD) and 165 stroke cases, and a subcohort of 1,375 subjects randomly selected from the Health Effects of Arsenic Longitudinal Study (HEALS) in Bangladesh, and 2) a cross-sectional study of 1,078 participants in the subcohort. We evaluated whether the association of arsenic exposure with CVD risk and carotid intima-media thickness (cIMT) differs by 360 single-nucleotide polymorphisms (SNPs) in 18 genes related to arsenic metabolism, oxidative stress, inflammation, and endothelial dysfunction. We found significant interactions of well-water arsenic with ICAM1 rs281432 (Padj = 0.0002) and VCAM1 rs3176867 (Padj = 0.035) in CVD risk after adjustment for multiple testing. These interactions were similar for stroke risk but weaker for CHD risk. We also found that NOS3 rs2853792 and SOD2 rs5746088 were significantly related to a reduced risk of CVD and CHD, and that MTHFR rs1801133 was related to a significantly increased risk of stroke. Three SNPs (rs10883790, rs11191442, and rs3740392) in AS3MT showed nominally significant interactions with both well-water arsenic and urinary creatinine-adjusted arsenic in cIMT. Our data provide novel evidence that the cardiovascular effects of arsenic exposure may vary with some common genetic variants in genes related to arsenic metabolism and endothelial dysfunction.
Poster Board #19

Prenatal inorganic arsenic (iAs) exposure in the CD-1 mouse leads to altered mammary gland development

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Affiliation: University of North Carolina-Chapel Hill

There are major public concerns regarding the effects of arsenic contamination on human health. The spectrum of exposures has led to endocrine disruption at levels ~ 150 times lower than EPA’s recommended levels of 10 ppb, and resulted in carcinogenesis at levels ≥42.5 ppm. The ability to disrupt the normal hormonal milieu makes endocrine regulated tissues such as the mammary gland a prime target for altered development. Our goal was to evaluate the morphological development of the gland at varied exposures of arsenic. Mammary glands were obtained from CD-1 female offspring on days 21, 28, 6 mos and 1yr, following a prenatal dose of sodium arsenite during gestation days 10-18. All glands were assigned a developmental score (1-4) using whole mounts and evaluated for histological changes. Scores at day 21 were reduced in the 10ppb group (1.77±0.26) yet significantly accelerated in the 42.5ppm group (3.15±0.24) compared to controls (2.19±0.19). At week 4, development in the 42.5 ppm and controls were comparable. However, 10ppb caused significant delays. By 6 mos, both groups exhibited abnormally advanced growth compared to controls. Histological assessment at this age also revealed that the 10ppb group demonstrated disorganized luminal epithelium with ductal papillary structures. Conversely, the 42.5 ppm group displayed a varied morphology that included sparse ductal structures, but multilayered luminal epithelium. These findings imply that early iAs exposure at both high and low doses can detrimentally alter the normal development of the mammary gland further predisposing it to later life disease.
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The Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Gómez Palacio, Mexico was recently established to better understand the impacts of prenatal exposure to inorganic arsenic (iAs). In the present study, we examined a subset (n=40) of newborn cord blood samples for microRNA (miRNA) expression changes associated with in utero arsenic exposure. Levels of iAs in maternal drinking water (DW-iAs) and maternal urine were assessed. Levels of DW-iAs ranged from below detectable values to 236 µg/L (mean=51.7 µg/L). Total arsenic in maternal urine (U-tAs) was defined as the sum of iAs and its monomethylated and dimethylated metabolites (MMAs and DMAs, respectively) and ranged from 6.2 to 319.7 µg/L (mean=64.5 µg/L). Genome-wide miRNA expression analysis of cord blood revealed 12 miRNAs with increasing expression associated with U-tAs. Transcriptional targets of the miRNAs were computationally predicted and subsequently assessed using transcriptional profiling. Pathway analysis demonstrated that the U-tAs-associated miRNAs are involved in signaling pathways related to known health outcomes of iAs exposure including cancer and diabetes mellitus. Immune response-related mRNAs were also identified with decreased expression levels associated with U-tAs, and predicted to be mediated in part by the arsenic-responsive miRNAs. Results of this study highlight miRNAs as novel responders to prenatal arsenic exposure that may contribute to associated immune response perturbations.
Day 1: Non-Competition Posters

Poster Board #23

Early vaginal opening and metabolic changes in female mice exposed to arsenic in utero

Karina F. Rodriguez, Yasmin Crespo-Mejias, Chang Liu, Erica K. Ungewitter Heather L. Franco, Barbara C. Nicol, Humphrey H.-C. Yao

Affiliation: NIEHS

Gestational exposure of rodents to arsenic causes tumors in ovary, adrenal, and liver. In this study we investigate whether exposure to arsenic during fetal life impacts the reproductive system from birth to adulthood. Pregnant CD-1 mice were exposed to sodium arsenite in drinking water at 0 (control), 10 ppb, and 42.5 ppm from embryonic day (E) 10 to 18, the window when the reproductive system develops. The exposed animals were analyzed before birth (E18) or allowed to reach adulthood. We found that arsenic exposed female offspring had increased body weight before birth. This increase in weight persisted to adulthood and was associated with significant increase of body fat and incident of glucose intolerance. Furthermore, when exposed females reached weaning age, they exhibited early onset of vaginal opening (indicator of puberty in mice). In addition, female offspring exposed to arsenic in utero exhibited abnormal progression of the estrous cycle with more time spent in proestrus and less in metestrus (P<0.05). Microarray analysis of gene expression profiles from ovaries of 3 week- and 4 week-old females uncovered significant differences between control and arsenic exposed groups. In conclusion, our findings reveal unexpected effects of in utero exposure to arsenic on the onset of vaginal opening, ovarian gene expression and metabolism in female mice. Furthermore, the negative impact of low dose arsenic exposure (10 ppb: EPA drinking water limit) renews the concerns on unpredictable nature of action of potential endocrine disruptors. This work was supported by NIH Intramural Research Fund.
Poster Board #25

Linda Valeri Brent Coull, Harvard School of Public Health, MA
Birgit Claus Henn, Harvard School of Public Health, MA
Molly L. Kile, Oregon State University, OR
Omar Sharif Ibn Hasan, Dhaka Community Hospital, Bangladesh
Quazi Quamruzzaman, Dhaka Community Hospital, Bangladesh
David C. Christiani, Harvard School of Public Health, MA
David C. Bellinger, Childrens Hospital Boston, MA
Robert O. Wright, Harvard School of Public Health, MA
Maitreyi Mazumdar, Boston Children's Hospital, Boston MA

Affiliation: Boston Children's Hospital

Arsenic, manganese and lead exposure are common in Bangladesh. We examined whether exposure to mixtures of arsenic, lead and manganese are associated with neurocognitive outcomes and whether there is evidence of interaction among metals. Umbilical cord blood samples were collected at delivery from 781 infants in Bangladesh. Whole blood arsenic, lead, and manganese were measured using ICP-MS. Bayley scale of infant development Third Edition (BSID-III) scores were measured at approximately 24 months of age. Associations of cognitive and motor composite scores with lead and manganese concentrations on the logarithmic scale, and arsenic categorized in tertiles, were modeled allowing for quadratic effect of ln-manganese and for interactions between metals. Mean (SD) cord blood levels for arsenic, lead, and manganese were 0.951 (1.905) µg/dl, 6.176 (6.75) µg/dl, and 12.575 (15.75) µg/dl, respectively. In adjusted multivariable regression models, lead, the second tertile of arsenic, and manganese were inversely associated with 24-month motor composite score (β=-0.71, p-val=0.01; β=-2.13, p-val=0.01; β=-1.25 , p-val=0.061, respectively). There was evidence of lead-arsenic interaction. Lead toxicity was increased among children in third tertile of arsenic exposure, compared to children in first tertile of arsenic (β=-0.33, p-val=0.006). Bangladeshi children have high exposure levels to arsenic, lead and manganese. We found evidence of interactions among these metal exposures, suggesting that joint exposure is synergistic. The interactions were non linear and would have been missed using main effects regression models.
Neural tube defects are serious birth defects that result from the failure of the neural tube to close in early gestation, resulting in life-long disabilities and varying degrees of paralysis in surviving infants. Arsenic induces neural tube defects in several animal models, but the potential of arsenic to cause neural tube defects in humans is unknown. Bangladesh is simultaneously experiencing an epidemic of arsenic poisoning as well as an epidemic of neural tube defects. To investigate whether these epidemics are related, we conducted a case-control study in rural Bangladesh that compared maternal arsenic exposure among infants with neural tube defects and age-and sex-matched controls. Our pilot study established a new birth defects surveillance programs in remote areas, and we enrolled 47 affected infants and their mothers, as well as 57 controls. Our preliminary data show that drinking water concentrations of from wells used by mothers in early pregnancy were higher in cases of neural tube defects than in controls.
Previous studies suggest an increased risk for spontaneous pregnancy loss in association with exposure to high levels of inorganic arsenic (iAs) in drinking water (>10 µg/L), but there has been little focus on mild-moderate exposures (<10 µg/L). To address this data gap we conducted a hospital-based case-control study in Timis County, Romania. We recruited women with incident spontaneous loss of 5-20 weeks gestation as cases (n=150), and women with ongoing pregnancies matched by gestational age as controls (n=150). Drinking water exposure was reconstructed using data gathered by physician-administered questionnaire and weighted by iAs concentrations determined in residential sources using hydride generation-atomic absorption spectrometry (HG-AAS). We measured a range of 0 to 175.1 µg/L (median=0.42, 90th %tile=9.39). The odds ratio (OR) and 95% confidence interval (95%CI) for loss was increased among smokers in a conditional logistic regression model assessing the interaction between iAs exposure and cigarette smoking (P=0.058), adjusted for gestational and maternal age, education and prenatal vitamin use. A 10 µg iAs/L increase in average residential drinking water concentration elicited a 76% increase in the odds for loss among smokers (OR 1.76, 95%CI 0.75-4.14), although no increase among non-smokers (OR 0.70, 95% CI 0.45-1.09). Similar patterns were detected using alternate iAs exposure indices. A dose-response was also indicated in which odds of loss were higher for heavier smokers. The results of our evaluation conducted in Timis County suggest that women exposed concurrently to cigarette smoke and mild-moderate drinking water iAs contamination are at higher risk for spontaneous pregnancy loss.
Day 2
Competition Entries – Day 2

Poster Board #2

Dharmendra S. Dheeman and Barry P. Rosen

Affiliation: Department of Cellular Biology & Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL

Title: Human AS3MT does not catalyze oxidative methylation

Although As(III) SAM methyltransferase (AS3MT) have been the subject of extensive experimental investigations, there are still considerable uncertainties concerning the catalytic mechanism and arsenical products during the multi-step methylation process. Here we have addressed these issues through an in vitro study of a synthetic construct of human AS3MT that is 100-fold more active than cDNA-expressed enzyme, along with mutants of the four conserved cysteine residues using endogenous reductants, Trx/TR/NADPH and GSH. C156S and C206S were unable to methylate As(III) or MAs(III). However, C32S and C61S were able to methylate MAs(III) but nearly inactive in As(III) methylation. These observations suggest that Cys156 and Cys206 are essential for As(III) and MAs(III) binding and methylation and point to the importance of presence of both Cys32 and Cys61 in methylation of As(III). In a homology model of hAS3MT with bound As(III) and SAM, Cys32 and Cys61 form a disulfide bond in a loop near the SAM-binding domain but distant from As(III), which is bound to Cys156 and Cys206. On SAM-binding, the loop containing Cys32 and Cys61 moves toward the bound As(III). These data point to functional equivalence of Cys32 and Cys61 in promoting As(III) binding and subsequent methylation by SAM. We propose that the role of Trx/TR is to re-reduce the Cys32-Cys61 disulfide bond. In addition, at short reaction times only MAs(III) is formed and remains bound to the enzyme, clearly showing that the reaction is not an oxidative methylation.

Supported by National Institutes of Health grant R37 GM55425
Poster Board #4

Lishi Xie, Dan-Ping Hu, Wen-Yang Hu, Gail S. Prins

Affiliation: Department of Urology, University of Illinois at Chicago

Arsenic modulation of human prostate stem/progenitor cells
Inorganic arsenic (iAs) exposure has been associated with prostate cancer. Increasing evidence suggests that cancer may originate from transformation of tissue stem cells. We hypothesize that iAs exposures can reprogram prostate stem cells resulting in the dysregulation of differentiation capability and transformation ability. Primary prostate epithelial cells (PrEC) were used and human prostate stem/progenitor cells were isolated using 3-D prostasphere (PS) culture. Treatment of iAs for 72 hours at 1 µM significantly increased stem cell% in PrEC cells but decreased it at 5 µM. A similar inverted U shape response was observed in PS formation assay, indicating a stem cell modification by iAs. Surprisingly, treatment of iAs increased apoptotic cells% in day 7 PS at both doses and increased apoptosis associated gene expression only at 5 µM. Further, 5 µM iAs treatment greatly increased the relative expression of stemness genes and decreased differentiation genes in day 7 PS, implying the reprogramming of stem cell self-renewal and differentiation. Finally, iAs treatment at 0.1, 1 and 5 µM increased pAkt/Akt ratio in day 7 PS in a dose-dependent manner. This was accompanied by accumulation of LC3-II, a sign of autophagic flux blockage, suggesting an early transformation event being initiated. We conclude that short term iAs exposure can reprogram normal prostate stem cells that leads to differentiation defects and predispose to transformation initiation. Chronic iAs exposure to prostate stem cells is under investigation to further clarify the iAs effects on cancer initiation and carcinogenic progression.
Altered control of cell cycle regulation is an important cellular response to exposure to environmental agents, particularly genotoxicants. A critical participant in this regulatory process is the dual specificity phosphatase Cdc25A, which is a member of the Cdc25 family of phosphatases. These phosphatases play a key role in cell-cycle progression by dephosphorylating and activating cyclin-dependent kinases. In response to treatment with environmental genotoxins, Cdc25A is subject to posttranslational modifications which contribute to its proteasome-mediated degradation. For many proteins, posttranslational modification is often required for their normal biological function. The most thoroughly studied of these posttranslational modifications is phosphorylation, which has been reported for Cdc25A. Here, we provide evidence for the first time that Cdc25A can be acetylated in vivo and directly interacts with the transacetylase ARD1. We further show that arsenic, an environmental genotoxicant, increases Cdc25A acetylation. Taken together, it is likely that Cdc25A is regulated by acetylation, which in turn may be related to a specific cellular response to DNA damage. We intend to further clarify the relationship between Cdc25A acetylation and its function in cell cycle regulation and genomic integrity. Together, our studies advance our understanding of the dynamics of Cdc25A modification in response to environmental challenge and DNA damage and ask whether, and to what extent, acetylation of Cdc25A contributes to genomic integrity surveillance. Since Cdc25A is frequently overexpressed in multiple types of cancer, our findings may also point to mechanisms that underlie carcinogenesis.
Prenatal arsenic exposure and the epigenome: An assessment of functional consequences of 5-methyl cytosine DNA methylation on gene expression in a pregnancy cohort in Mexico

The Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Gómez Palacio, Mexico was established to better understand the impacts of prenatal exposure to inorganic arsenic (iAs) on newborn health. In the present study, we examined a subset of newborn cord blood samples for 5-methyl cytosine methylation changes in cord blood leukocytes and compared these to functional changes in gene expression, both in relationship to in utero arsenic exposure as well as at baseline. Arsenic-associated changes in DNA methylation were assessed across 424,935 sites at 6 different genomic regions, representing 20,520 genes. The methylation changes were compared to gene expression levels for more than 18,761 genes. We find that genome-wide arsenic-independent patterns of methylation were associated with gene expression levels. Arsenic-associated methylation was identified for 360 probes across the genome with a diminished proportion of differentially methylated probes occurring across the 3 untranslated region. While some patterns of arsenic-associated methylation were associated with gene expression, in general DNA methylation was not predictive of expression changes. Promoter region analysis identified that genes with arsenic-associated differential methylation patterns were enriched for specific transcription factor binding sites, including the Glucose Transporter Enhancer Factor (HDBP). Results of this study highlight the complexity of the relationships between contaminant-associated DNA methylation patterns and functional changes in gene expression.
Human AS3MT does not catalyze oxidative methylation

Although As(III) SAM methyltransferase (AS3MT) have been the subject of extensive experimental investigations, there are still considerable uncertainties concerning the catalytic mechanism and arsenical products during the multi-step methylation process. Here we have addressed these issues through an in vitro study of a synthetic construct of human AS3MT that is 100-fold more active than cDNA-expressed enzyme, along with mutants of the four conserved cysteine residues using endogenous reductants, Trx/TR/NADPH and GSH. C156S and C206S were unable to methylate As(III) or MAs(III). However, C32S and C61S were able to methylate MAs(III) but nearly inactive in As(III) methylation. These observations suggest that Cys156 and Cys206 are essential for As(III) and MAs(III) binding and methylation and point to the importance of presence of both Cys32 and Cys61 in methylation of As(III). In a homology model of hAS3MT, with bound As(III) and SAM, Cys32 and Cys61 form a disulfide bond in a loop near the SAM-domain cysteine residues but distant from As(III), which is bound to Cys156 and Cys206. On SAM-binding, the loop containing Cys32 and Cys61 moves toward the bound As(III). These data point to functional equivalence of Cys32 and Cys61 in promoting As(III) binding and subsequent methylation by SAM. We propose that the role of Trx/TR is to reduce the Cys32-Cys61 disulfide bond. In addition, at short reaction times only MAs(III) is formed and remains bound to the enzyme, clearly showing that the reaction is not an oxidative methylation.
Poster Board #12

Jiaojiao Li Barry Rosen

Affiliation: Herbert Wertheim College Of Medicine Florida International University

Role of Glutathione S-transferase in Arsenic Methylation

Arsenic is carcinogenic and ubiquitous in human environments. Methylation and glutathione conjugation are two reactions that are involved in phase II detoxification of xenobiotics. As(III) methyltransferase (AS3MT or ArsM) converts inorganic arsenic into organic products, which is a common means to detoxify arsenic. Arsenic-GSH conjugates are proposed to be substrates both for arsenic methyltransferase and for arsenic detoxification pumps such as MRP2. However, to date, it is not known whether arsenic-GSH conjugate formation is enzyme-catalyzed. We hypothesize glutathione S-transferases (GSTs) catalyze As(III) glutathionylation. In this poster I will describe our efforts to identify if human GSTs accelerate formation of As(GS)3. The rate of binding of As(OH)3 and As(GS)3 in the presence or absence of human GST Pi will be compared using changes in tryptophan fluorescence of a single-tryptophan containing enzyme or by kinetic assays analyzed by HPLC-ICP-MS. Supported by NIH grant R37 GM55425
Poster Board #14

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Arsenic is carcinogenic and ubiquitous in human environments. Methylation and glutathione conjugation are two reactions that are involved in phase II detoxification of xenobiotics. As(III) methyltransferase (AS3MT or ArsM) converts inorganic arsenic into organic products, which is a common means to detoxify arsenic. Arsenic-GSH conjugates are proposed to be substrates both for arsenic methyltransferase and for arsenic detoxification pumps such as MRP2. However, to date, it is not known whether arsenic-GSH conjugate formation is enzyme-catalyzed. We hypothesize glutathione S-transferases (GSTs) catalyze As(III) glutathionylation. In this poster I will describe our efforts to identify if human GSTs accelerate formation of As(GS)3. The rate of binding of As(OH)3 and As(GS)3 in the presence or absence of human GST Pi will be compared using changes in tryptophan fluorescence of a single-tryptophan containing enzyme or by kinetic assays analyzed by HPLC-ICP-MS.
Poster Board #16

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Arsenic is the most ubiquitous environmental toxin, entering the biosphere primarily from geochemical sources and anthropogenic activities. Microorganisms play an important role in biogeochemical cycling of arsenic by biotransformation of inorganic arsenic into organic arsenic species and degradation of organoarsenicals back to inorganic arsenic. ArsI is a microbial non-heme ferrous-dependent dioxygenase family enzyme that transforms toxic methylarsonious acid [MAs(III)] to less toxic inorganic arsenite [As(III)] by C-As bond cleavage. An ArsI orthologue from the thermophilic bacterium Thermomonospora curvatta was expressed, purified and crystallized. The crystals were diffracted up to 1.46 Å resolution. The structure was determined by Single Anomalous Dispersion (SAD) method using Ni(II) as an anomalous scatter. ArsI is a monomer with two domains; each domain consists of βαβββ secondary structural motifs. The eight β-sheets that surround a metal ion resemble a β-barrel motif or cupin fold. There are two binding sites in ArsI, a metal binding site and a organoarsenical binding site. The structures of ArsI with Ni(II) and Co(II) are clearly show the metal binding site. The metal is bound with an octahedral geometry that includes three coordinations from the protein (Gln5, His62 and Glu114) and three from water molecules. The arsenic binding residues are in a floppy region and are not visible in the crystal structure. Co-crystallization of ArsI with organoarsenical substrates is in progress. Supported by National Institutes of Health grant R37 GM55425 to bpr. We acknowledge the synchrotron beam lines at ALS, Berkeley and APS, Chicago.
Arsenic methylation is simultaneously a detoxification process and a pathway of biotransformation of inorganic arsenic into more carcinogenic species (MAs(III) and DMAs(III)). The reaction is catalyzed by the enzyme As(III) S-adenosylmethionine (SAM) methyltransferase, which is termed ArsM in unicellular organisms and AS3MT in multicellular organisms, including humans. ArsMs and AS3MTs have four conserved cysteine residues. We used a structural biology approach to investigate the role of the conserved cysteines. New crystal structures of CmArsM from the thermophilic eukaryotic alga Cyanidioschyzon merolae exhibit a novel disulfide bond between two of the conserved cysteines, Cys44 and Cys72 when trivalent aromatic arsenicals phenylarsenite (PhAs(III) or PAO) (1.80 Å; pdb ID 4KW7) or reduced roxarsone (Rox(III)) (2.38 Å; pdb ID 4KU9) are bound. When SAM is bound, the N-terminal helix containing Cys44 and Cys72 moves approximately 6.5 Å in the direction of the As(III) binding domain, which is composed of the other two conserved cysteines, Cys174 and Cys224. In the SAM-bound structure a disulfide bond is observed between conserved cysteines Cys72 and Cys174. A model of the reaction pathway involving a disulfide bond cascade will be discussed. Supported by National Institutes of Health grant R37 GM55425 to B.P.R. We acknowledge the synchrotron beam lines at ALS, Berkeley and APS, Chicago for the data collection.
Poster Board #20

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ArsH: a trivalent organoarsenical oxidase

Organoarsenicals are produced by microorganisms and introduced anthropogenically as herbicides and antimicrobial growth promoters for poultry and swine. Nearly every prokaryote has an ars (arsenic resistance) operon with a variable number of genes. Some ars operons have an arsH gene encoding an atypical flavodoxin. Purified ArsH has a non-canonical FMN binding site, catalyzes oxidation of NADPH and can generate H2O2. ArsH genes are widely distributed in bacteria and also found in fungi, plant and archaea. The role of ArsH in arsenic resistance has been unclear. In this study, we demonstrate that ArsH is an organoarsenical oxidase that detoxifies trivalent methylated and aromatic arsenicals by oxidation to pentavalent species. E. coli, which does not have an arsH gene, is sensitive to the trivalent forms of herbicide MSMA (MAs(III)), antimicrobial growth promoter Roxarsone (Rox(III)), and phenylarsine oxide (PhAs(III)). In contrast, Pseudomonas putida, which has two chromosomally-encoded arsH genes, is highly resistance to trivalent organoarsenicals. A derivative of P. putida with both arsH genes deleted is sensitive to MAs(III), PhAs(III) and Rox(III). To examine the function of ArsH, cloned P. putida arsH was expressed in the arsenic hypersensitive E. coli strain AW3110, where it conferred resistance to MAs(III), PhAs(III) and Rox(III). The cells oxidized these trivalent organical arsenicals to pentavalent species in the order PhAs(III)>Rox(III)>MAs(III). Similar results were obtained in vitro with purified ArsH. These results suggest that ArsH catalyzes biotransformation and detoxification of environmental methyl and aromatic arsenicals. Supported by NIH Grant R37 GM55425
Poster Board #22

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Methylated arsenicals are more toxic than inorganic forms of arsenic. Arsenic methyltransferase (AS3MT) is responsible for the biotransformation of arsenic in the human body. It is anticipated that toxicity of arsenic can be lowered by inhibiting AS3MT enzyme. A real-time assay for methylation of arsenic is necessary to realize this aim. Fluorescence assays are sensitive and are easily scalable for high-throughput approaches. Here, a real-time tryptophan assay for binding of arsenicals has been developed with the AS3MT orthologue Chlamydomonas ArsM (CrArsM), which has high sequence identity to AS3MT. CrArsM is a very active enzyme. A single tryptophan was engineered in CrArsM near the As(III) binding site. Intrinsic protein fluorescence of this single-tryptophan enzyme responds to binding of As(III) and MAAs(III) with substantial quenching, allowing for determination of the kinetics of substrate binding and the effect of cysteine substitutions. CrArsM has 16 cysteine residues, but only cysteines at positions 46, 74, 170 and 220 of CrArsM are conserved in other ArsM/AS3MT orthologues. In vitro studies shows that mutation of the nonconserved cysteines is tolerated for biotransformation of As(III). In contrast, mutation of Cys46, 74, 170 or 220 results in loss of As(III) methylation. Fluorescence studies with CrArsM show that rate of binding of arsenic glutathione complex As(GS)3 to CrArsM is much faster than As(III). These results support the hypothesis that As(GS)3 is the physiological substrate of CrArsM. Supported by NIH grant R37 GM55425.
Arsenic is the most wide-spread environmental toxin. Substantial amounts of pentavalent organoarsenicals have been utilized as herbicides such as MSMA (monosodium methylarsonic acid or MA(V)) and as growth enhancers for animal husbandry such as roxarsone (4-hydroxy-3-nitrobenzenearsonic acid or Rox(V)). These undergo environmental degradation to more toxic inorganic arsenite (As(III)), which leads to environmental pollution. We have discovered a novel pathway of degradation of MSMA to As(III) by microbial communities involving sequential reduction to methylarsinous acid (MA(III)) by one bacterial species and demethylation from MA(III) to As(III) by another. Recently we identified the gene responsible for MA(III) demethylation from an environmental MA(III)-demethylating isolate, Bacillus sp. MD1. This gene, termed arsI, is in an arsenic resistance (ars) operon and encodes a non-heme iron-dependent dioxygenase. Heterologous expression of ArsI conferred MA(III)-demethylating activity and MA(III) resistance to an arsenic-hypersensitive strain of Escherichia coli, demonstrating that MA(III) demethylation is a detoxification process. Purified ArsI catalyzes Fe2+-dependent MA(III) demethylation. In addition, ArsI cleaves the C-As bond in trivalent roxarsone (Rox(III)) and other aromatic arsenicals, strongly suggesting that in the environment pentavalent aromatic arsenicals also undergo two-step pathway of sequential reduction and ArsI-catalyzed C-As bond cleavage, in analogy with the demethylation of the MA(V) herbicide by a microbial community. ArsI homologues are widely distributed in prokaryotes, and we propose that ArsI-catalyzed organoarsenical degradation has a significant influence on the arsenic biogeochemical cycle. This is the first report of a molecular mechanism for organoarsenic degradation by a C-As bond lyase.
Inhibition of Microglia Pro-inflammatory Response to LPS by Inorganic Arsenite

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Inorganic arsenic (iAs) exposure alters immune system functioning resulting in decreased ability to adequately respond to challenge. In the brain, microglia serve as resident immune cells to remove pathogens, aberrant proteins, and cellular debris and may be similarly affected by iAs. BV-2 microglia cells exposed to iAs (10µM; 24hrs) increased mRNA levels for the pro-inflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-1α by 2-5-fold. When challenged with lipopolysaccharide (LPS, 100ng/6hrs), elevations in inducible NO synthase (iNOS), IL-1α, IL-1β, IL-6, and TNF-α mRNA levels were significantly blunted by iAs. At 2.5 µM iAs, a 24 hr exposure increased IL-1α and TNF-α (1.5-2fold), blocked LPS induction of iNOS with no effect on pro-inflammatory cytokines. iAs (2.5µM) exposure for 7-21 days blunted the response to LPS. When a comparable response was examined in 6-week-old, male mice exposed to iAs (42.5 ppm/5 weeks) in drinking water, basal levels of pro-inflammatory cytokines and iNOS in the hippocampus and frontal lobe remained similar to controls. In response to LPS (0.1 mg/kg) elevations in IL-α, IL-1β and IL-6 were significantly blunted at 3hrs. Thus, while arsenic exposure elevated pro-inflammatory cytokine mRNA levels in isolated microglia this was not observed in vivo. However, the similar pattern of a blunted immune response in both systems suggests that the systemic immune suppression associated with arsenic exposure translates to the brain. Given the dynamic and critical role for microglia in brain development and repair, this may represent a cellular mechanism by which arsenic exposure compromises the CNS.