Arsenical Metabolism and Dosimetry: The Bridge Between Arsenic Exposure and Health Effects

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LTG 1 Poster 08

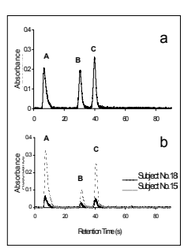
Science Questions

- What are the pathways for metabolism of arsenicals?
- Do specific arsenic-containing metabolites account for some of the health effects commonly attributed to inorganic arsenic?
- What are the genetic and environmental determinants of the capacity to metabolize arsenic?
- Can pharmacokinetic (PK) modeling be used to develop integrated models for arsenic metabolism that may be linked with pharmacodynamic models?

Research Goals

- Develop and apply analytical methods for identification of metabolites of inorganic arsenic
- Examine metabolism and disposition of arsenic, particularly the role of arsenic (+3 oxidation state) methyltransferase
- Integrate data on metabolism and disposition through development of pharmacokinetic and pharmacodynamic models for mouse and human

Analytical Optimization



a. Optimization of cysteine reduction method allows quantitation of inorganic (A), methyl (B), and dimethyl (C) by hyride generation-atomic absorption spectrometry at 8-20 pg limit of detection.
b. Method applied to detection of arsenicals in exfoliated urinary bladder epithelial cells.

Findings and Conclusions

Development and refinement of analytical methods permits the quantitation of arsenicals in biological samples. Development of new mass spectrometric methods permits detection of thioarsenical metabolites. Improved analytical methods provide dosimetric data for model development and mode of action studies.

Studies of the enzymatic basis of arsenic methylation provide data for model development and refinement and identify metabolites of potential importance in mode of action studies. These studies also elucidate genotype-phenotype relations in human populations.

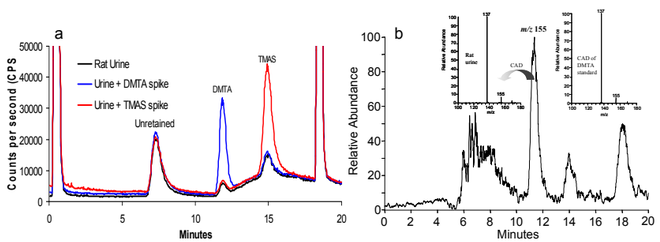
Studies of metabolism and disposition in wildtype and As3mt knockout mice provide additional data for model development and refinement. Studies of genotype-phenotype relations in knockout mice may aid in mode of action studies.

Data from subchronic pharmacokinetic studies indicate that models for As metabolism and disposition need to include mechanisms for organ-specific accumulation of some arsenicals and that urinary metabolite profiles are not necessarily reflective of target tissue dosimetry.

The As human PBPK model can be used to examine the relationship between target tissue dose of arsenic metabolites and response in exposed human populations.

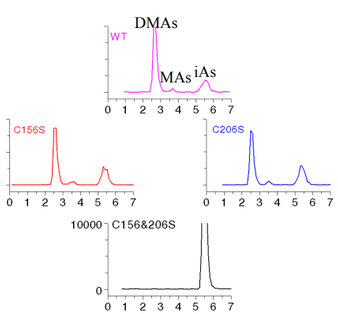
Methods/Approach

Identifying Novel Metabolites of Inorganic Arsenic

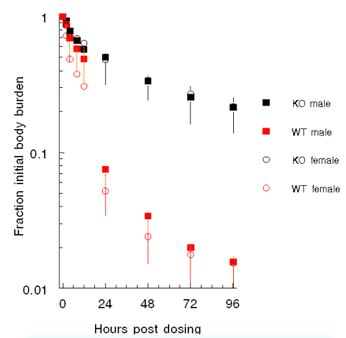


a. LC-ICP-MS chromatograms of rat urine and rat urine spiked with DMTA (dimethylthioarsenic) and TMAS (trimethylarsenic sulfide). Coelution of spikes and unknowns identifies DMTA and TMAS as urinary metabolites.
b. The identify of DMTA in urine was confirmed by ESI-MS.

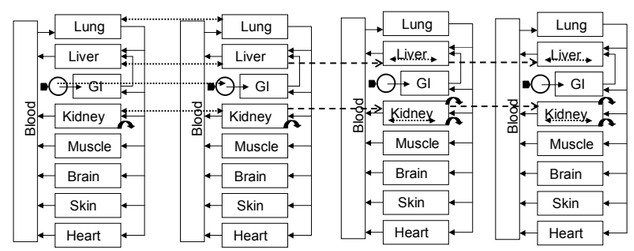
Genotype- Phenotype Relations in Arsenic Metabolism



Structure-activity studies with recombinant human wild type (WT) arsenic (+3 oxidation state) methyltransferase (AS3MT) shows that WT enzyme catalyzes the conversion of inorganic As (iAs) to methyl (MAs) and dimethyl (DMAs) arsenic. Site-directed mutagenesis to replace cysteine 156 or cysteine 206 with a serine does not affect AS3MT's catalytic activity. However, when both cysteines are replaced with serines, catalytic activity is lost.



Arsenic methylation and the clearance of orally ingested arsenic. As3mt knockout mice (KO) and C57BL/6 wildtype (WT) mice received single oral doses of 0.5 mg of As as arsenate (iAs^V) labeled with $^{73}iAs^V$ and were radioassayed in a whole body counter for 96 hours post dosing. Whole body retention differed markedly on the basis of genotype; loss of the capacity to methylate arsenic slows the rate of whole body clearance.

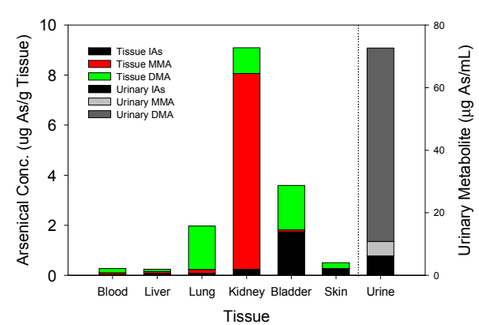


A schematic of the overall PBPK model for inorganic As with sequential reduction of As(V) to As(III) and methylation to MMA and DMA. Reduction and metabolism of inorganic arsenic, MMA, and DMA are modeled in the liver and kidney compartment. Rates of MMA and DMA produced from inorganic As metabolism are calculated in the As submodel and added to the MMA and DMA submodels, respectively. Rate of DMA formation from MMA metabolism is calculated in the MMA model and added to the liver and kidney compartments. Levels of MMA(III) and As(III) in liver and kidney are introduced to their respective equations for the noncompetitive inhibition of As and MMA methyltransferase activities, respectively.

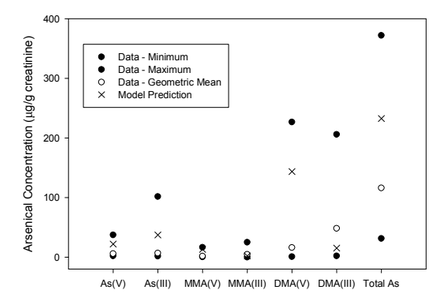
Inorganic Arsenic Human PBPK Model

- The overall model consists of sequential interconnected individual PBPK models for As(V), As(III), monomethylarsenic acid (MMA), and dimethylarsenic acid (DMA).
- Metabolism of inorganic arsenic in liver was described as a series of reduction and oxidative methylation steps incorporating the inhibitory influence of metabolites on methylation.
- Novel features of this model are: (1) an updated description of metabolism consistent with recent biochemical data on the mechanism of arsenic methylation, (2) extensive use of human data (*in vivo* and *in vitro*) for parameter estimation such that only parameters sensitive to the shape of the urinary excretion curve are optimized, and (3) the ability to predict formation and urinary excretion of trivalent methylated arsenicals.

Disposition Studies Support Model Development and Validatiion



Subchronic (13 weeks) exposure of mice to 50 ppm As(V) in drinking water results in tissue-specific patterns of metabolite distribution and accumulation.



Comparison of model predictions (X) and data (minimum and maximum are filled circles, geometric mean is open circle) for pentavalent and trivalent arsenicals excreted in urine of subjects (n=21) in central Mexico exposed to an average arsenic drinking water concentration of 117 µg/L

Impact and Outcomes

- Elucidation of pathways of arsenic toxicity provides mode of action information for use by OW in its As risk assessment
- Identification of new metabolites of inorganic As which are likely to be mediators of toxic or carcinogenic effects associated with the chronic exposure to inorganic As are vital to design of appropriate mode of action studies.
- Results of genotype-phenotype relationships can be used to strengthen risk assessment for inorganic arsenic by incorporating information about genetic susceptibility
- Rodent and human PBPK models now available can be used as tools for experimental planning, particularly for mode of action studies, and for various risk assessment applications, including linkage with BBDR models

Future Directions

- The As3mt knockout mouse can be used as a model system to investigate more precisely the role of metabolism in arsenic-induced toxicity
- Ongoing metabolism studies are designed to support development and refinement of pharmacokinetic models
- Metabolism studies also support design of mode of action studies
- PBPK models for arsenic metabolism in both humans and laboratory animals will be used to
  - evaluate the impact of variability in arsenic metabolism due to factors such as age and genetic polymorphisms in *As3mt*
  - correlate measures of internal dose with cancer and non-cancer health effects under various exposure scenarios
  - evaluate the potential for exposure reconstruction using biomarker data in collaboration with exposure modelers

Researchers Involved

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