Mode of action research of arsenic-induced tumors and toxicity

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Contributing Organizations: NHEERL/ORD, NERL and UNC Chapel Hill

LTG 1 Poster 07

Science Questions

- What is (are) the MOA(s) of arsenic-induced carcinogenicity and toxicity?
- Which arsenic species is (are) the cause of adverse health effects?
- With respect to carcinogenicity, does arsenic act in a genotoxic or nongenotoxic mode at low concentrations?

Research Goals

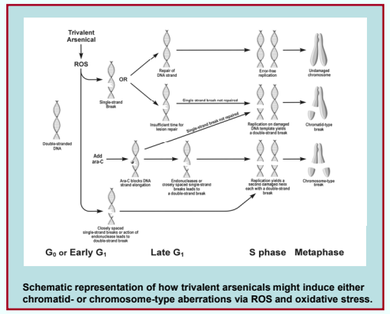
- To describe a mode of action for arsenic-induced carcinogenicity, one has to identify the key events that describe the process and link them to the pharmacokinetics of the arsenical of toxicological interest. At the present time, several key events that may be common across different species, organs and adverse health effects are (a) binding to protein sulfhydryl groups, (b) ROS generation and associated oxidative stress, (c) DNA methylation changes, (d) alterations of toxicity and carcinogenicity pathways and (e) chromosomal structural and numerical alterations.
- Ultimately, the data can provide information for the development of a Biologically-Based Dose-Response (BBDR) model. A BBDR model for arsenic is needed for future Agency risk assessments of inorganic arsenic and organic arsenical pesticides.

Methods Approach

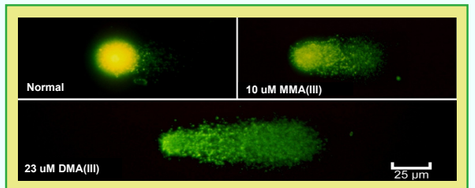
Three Ways Arsenic Interacts with Biological Molecules

- Trivalent arsenicals bind to sulfhydryl groups of peptides and proteins
- Generation of free radicals, causing oxidative stress, (arsenic radicals, ROS, RNS, 8-OHdG…)
- Alters DNA methylation state
  ↑↓ DNA methytransferases enzymes,
  ↓ S-adenosylmethionine (SAM) pools

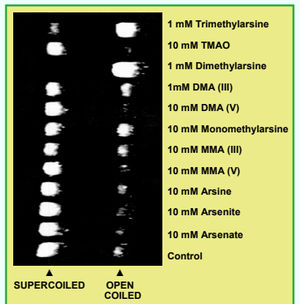
Results and Conclusions



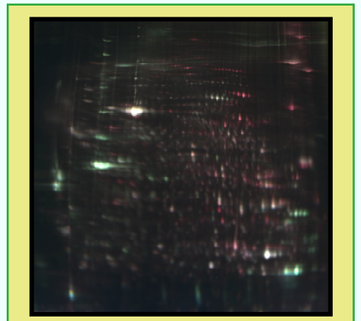
Schematic representation of how trivalent arsenicals might induce either chromatid- or chromosome-type aberrations via ROS and oxidative stress.



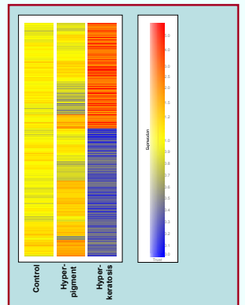
The comet assay shows the ability of MMA(III) and DMA(III) to fragment DNA in cell cultures.



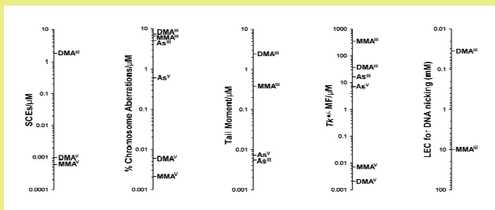
Agarose gel electrophoses of pBR 322 plasmid DNA after incubation with 11 different arsenicals for 1 hour at 37°C at pH 8.0.



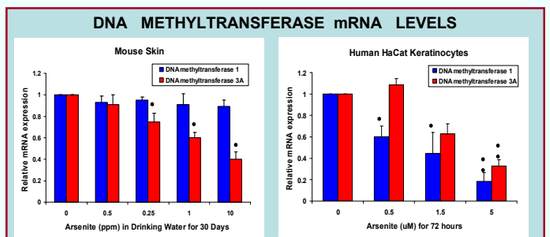
Representative 2-D DIGE gel image of CyDye-labeled proteins from mouse urinary bladder treated with 85 ppm arsenite for 30 days



Genomic study of control, hyperpigmented and hyperkerotic arsenic exposed human skin from the Inner Mongolia region of China. There are ~900 significant genes in hyperkeratosis group.

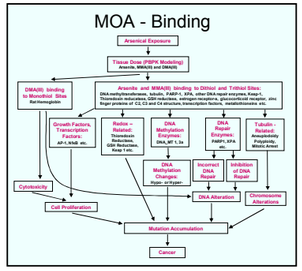


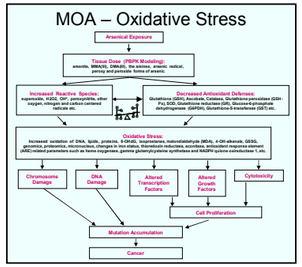
The in vitro gentoxicity of six different forms of inorganic and methylated arsenic (arsenate, arsenite, MMAV, MMAIII, DMAV and DMAIII) has been determined in seven different in vitro genotoxicity/mutation systems (sister chromatid exchange, chromosome aberrations, comet assay, DNA nicking, prophage induction, Ames mutagenicity and mutation in mouse lymphoma cells). Briefly, MMAIII and DMAIII are nearly always the most potent genotoxic arsenicals. They induce DNA damage and chromosome breakage, but are marginal point mutagens at best.

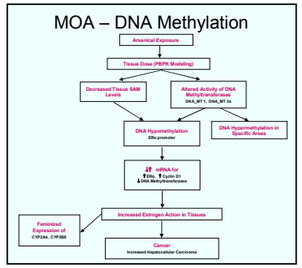


Arsenite exposures decreased DNA methyltransferase mRNA levels in mouse skin in NaCat keratinocytes in vitro. These studies show that inhibition of DNA methylation enzymes by arsenicals occurs and could account for some of the known genotoxicity of arsenicals.

Impact and Outcomes







These completed and ongoing MOA studies can be used to assist in the development of future BBDR model(s) which will provide a more scientific and defendable basis for regulation of the cancer and noncancer health risks of exposure to inorganic arsenic and organic arsenicals. MOAs based on binding, oxidative stress and altered DNA methylation are shown linking arsenic exposure to tumor outcome. These MOAs could occur simultaneously or sequentially.

- The Office of Water used ORD research in promulgating the lower MCL of 10 ppb for arsenic in drinking water in 2001. This standard was enforced in 2006.
- The Office of Pesticide Programs used ORD arsenic research in support of its Dimethyl Arsenic Acid Mode of Action Document submitted to and supported by the OPP Science Advisory Board.

Future Directions

- Several possible MOAs for arsenic will be pursued experimentally, particularly the oxidative stress MOA. Integration of experimental and modeling efforts based on existing as well as ongoing work will be used to contribute to the development of BBDR models particularly in the sense of a two dimensional dose- and time- dependent data sets of key intermediate events.
- Particular MOAs can be combined with a PBPK model of speciated arsenicals for those major tissues of regulatory interest to the Agency – lung, urinary bladder and skin. Combining a PBPK and a MOA model into a BBDR will provide a stronger, scientific basis for risk assessments.

Researchers Involved

Kirk Kitchin, Andrew Kligerman, Yue Ge, Witold Winnik, James Allen, William Ward, Douglas Wolf, Susan Hester, Sheau-Fung Thai, David Thomas, Judy Mumford, David DeMarini, Don Delker, Gene Ahlborn, Kathryn Bailey, Pedro Ortiz and Paul Andrewes NHEERL, Jack Creed, US EPA and Mirek Styblo, UNC. A large number of people in the arsenic BBDR research group were involved in brainstorming, critiquing and synthesizing various possible key events into the version of MOAs shown in this poster.

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