Current Understanding of Mechanisms Underlying Arsenic-Induced Developmental Toxicity

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Conflict of Interest Statement

- I have no conflicts of interest to report.
Objectives

1. Describe the health impacts of *in utero* exposure to arsenic on early and later-in-life health
2. Discuss epigenetic mechanisms that may link such developmental origins of health and disease
3. Highlight methods to assess dose-response of epigenetic effects
4. Highlight sexual dimorphism in arsenic-induced health effects
Inorganic Arsenic Continues to Contaminate the Water of Millions Around the Globe

Hundreds of millions of individuals are exposure to high levels of arsenic in drinking water
Arsenic is associated with both cancer and non-cancer endpoints.

- Classified as Group 1 Carcinogen by the International Agency for Research on Cancer (IARC)
- Chronic exposure results in many cancers: skin, bladder, lung, liver, prostate, and kidney
- Exposure is associated with non-cancer endpoints: neurological disorders, reproductive effects, cardiovascular disease, diabetes
Early Life Health Effects of Inorganic Arsenic

- Birthweight (growth restriction)
- Immune dysfunction-increased risk for infections
- Increased mortality
- Cognitive impairments in children
Early Life Exposures Associated with Later in Life Health Effects

Mice

- CD1 mice
- Hepatocellular carcinomas
- Prenatal exposure to adulthood
- Permanent changes in gene expression

Humans

- Cancer mortality
- Bladder cancer
- Laryngeal cancer
- Lung cancer
- Kidney cancer
- Liver cancer
- Other cancer
- Noncancer mortality
- Bronchiectasis
- Other COPD
- Acute myocardial infarction
- Chronic renal disease
- Other noncancer

- SMR = 18.1 (95% CI: 11.0, 27.4)"**, SMR = 8.1 (95% CI: 3.5, 16.0)**, SMR = 7.0 (95% CI: 5.0, 9.8)***, SMR = 3.5 (95% CI: 2.1, 4.5)***, SMR = 2.5 (95% CI: 1.0, 5.7)***, SMR = 1.2 (95% CI: 1.1, 1.3)**

- SMR = 18.4 (95% CI: 16.3, 20.4)***, SMR = 2.0 (95% CI: 1.7, 4.5)**, SMR = 2.1 (95% CI: 1.3, 2.5)**, SMR = 2.0 (95% CI: 1.5, 2.8)**, SMR = 0.9 (95% CI: 0.3, 1.0)**

- Smith et al. 2012

Early Life Exposures Associated with Later in Life Health Effects

Mice

- CD1 mice
- Prenatal exposure
- Permanent changes in gene expression

Humans

- Hepatocellular carcinomas
- Mice
- Humans

Arsenic is a model contaminant for the study of the developmental origins of health and disease hypothesis (DoHAD)

Waalkes, M. et al. 2004

Xie, Y. et al. 2007

Cancer

Non-cancer

M Waalkes

A Smith

Smith et al. 2012
Early Life Exposures Associated with Later in Life Health Effects

Biomarkers:
Epigenetic, Genomic, Proteomic, Metabolomic, Inflammatory responses,

Early Life Exposures Associated with Later in Life Health Effects

Biomarkers:
- Epigenetic
- Genomic
- Proteomic
- Metabolomic
- Inflammatory responses
- Cellular and molecular machinery

Which of these mechanisms is relevant to developmental toxicity?

What is the biological chain of events linking arsenic to developmental toxicity?

Do Epigenetic Mechanisms Underlie Health Effects Associated with Early Life Exposure?

- Gene expression
- Histone modification
- CpG methylation
- miRNA expression

Gene expression

SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety
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Prenatal Arsenic Exposure is Associated with Detrimental Birth Outcomes: Lower Birthweight

Mexico. Laine et al. EHP. 2015 Feb; 123 (2); Arsenic range: 0-236 ppb
United States, Oklahoma. Henn et al. EHP. 2016 Aug; 124 (8); Maternal and umbilical cord blood arsenic, 1.0–2.3 and 1.8–3.3 μg/L
Prenatal Arsenic Exposure is Associated with Detrimental Birth Outcomes: Lower Birthweight
Elevated Levels of Inorganic Arsenic in Water in Mexico

Laine et al, 2014 EHP

G Garcia-Vargas

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Impact of CpG Methylation on Gene Expression
Assessed Genome-wide Using the Illumina 450k Assay
The Imprinted Gene KCNQ1 has Altered CpG Methylation in Relationship to Prenatal Arsenic Exposure

KCNQ1: Potassium voltage-gated channel, KQT-like subfamily, member1

KCNQ1 differential methylation has been associated with gestational age. Lee et al 2012, Kwak et al Horm Res Paediatr 2010;74:333–33

Periconceptual exposure to famine during the Dutch Hunger Winter (1944-45) linked to decreased methylation of Insulin-like growth factor 2 (Heijmans, PNAS 2008)
Imprinted Genes Are Tied to Size at Birth

Imprinted genes defy rules of Mendelian genetics with their expression tied to the parent from whom each allele was inherited.

Genomic tug-of-war between mothers and fathers over the use of maternal resources by the fetus.

Jirtle and Weidman, American Scientist, March 2007
Imprinted Genes Are Tied to Size at Birth
Imprinted Genes Are Tied to Size at Birth

Critical genes that REGULATE fetal growth are targeted for arsenic-associated DNA methylation.
Imprinted Genes-a Link Between Preconception Arsenic and Health Outcomes

**Effects of Preconception and In Utero Inorganic Arsenic Exposure on the Metabolic Phenotype of Genetically Diverse Collaborative Cross Mice**

Rebecca C. Fry, Kezia A. Addo, Timothy A. Bell, Christelle Douillet, Elizabeth Martin, Miroslav Styblo, and Fernando Pardo-Manuel de Villena

Sex-dependent effects of preconception exposure to arsenite on gene transcription in parental germ cells and on transcriptomic profiles and diabetic phenotype of offspring

Abhishek Venkatratnam, Christelle Douillet, Brent C Topping, Qing Shi, Kezia A Addo, Folami Y Ideraabdullah, Rebecca C Fry, Miroslav Styblo

Affiliations + expand
PMID: 33145626 DOI: 10.1007/s00204-020-02941-w

Applying a Benchmark Dose Approach to Epigenetic Datasets

- Mathematically model the relationship between quantified exposures (doses) and the change in the incidence or severity of a response
- Use various curve fits to model dose-response relationships
- Identify values to use in risk assessment calculations from the best fitting model curve

LOAEL: Low Observed Adverse Effect Level
NOAEL: No Observed Adverse Effect Level
BMD: Benchmark Dose
BMDL: Benchmark Dose Lower Bound
Applying a Benchmark Dose Approach to Epigenetic Datasets

\[ \text{BMDL} = 55 \text{ ppb} \]

\[ \text{BM} = 86 \text{ ppb} \]

\[ \text{CpG sites (n=1629)} \]
\[ \text{miRNAs (n=12)} \]
\[ \text{mRNAs (n=96)} \]
\[ \text{Proteins (n=14)} \]

\[ \text{BMD (U-tAs, µg/L)} \]
\[ \text{BMDL (U-tAs, µg/L)} \]
Applying a Benchmark Dose Approach to Epigenetic Datasets

Identify concentrations at which contaminants induce epigenetic effects... with biological plausibility as causal factors in infant outcomes
Do Epigenetic Mechanisms Underlie Health Effects Associated with Early Life Exposure?
Early Life Health Effects of Inorganic Arsenic

- Birthweight (growth restriction)
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The overall average drinking water As concentration was 5.2 µg/L (range 0.01–67.5 µg/L).
Epigenetic Effects of Prenatal iAs Exposure: Potential Reprogramming by iAs Early in Life

~22 bp

miRNA

Degradation

Block Translation
Epigenetic Effects of Prenatal iAs Exposure: Potential Reprogramming by iAs Early in Life

Innate and adaptive immune response genes are enriched:

Toll like receptors: TLR5, TLR9
Interferon gamma pathway

Environmental and Molecular Mutagenesis 55:196–208 (2014)
miRNAs REGULATE the expression of innate/adaptive immune response genes

Potential role in response to infectious agents
Do Epigenetic Mechanisms Underlie Health Effects Associated with Early Life Exposure?

- Gene expression
- Histone modification
- CpG methylation
- miRNA expression
- Gene expression
Summary

- Epigenetic mechanisms may tie early life arsenic exposure to early and later in life health outcomes
  - miRNAs
  - CpG methylation
- The placenta is a key target organ for developmental exposure to arsenic
- Preconception arsenic exposure is tied to offspring health in mice
References

- Laine, Jessica E. et al. 2015. Maternal Arsenic Exposure, Arsenic Methylation Efficiency, and Birth Outcomes in the Biomarkers of Exposure to ARsenic (BEAR) Pregnancy Cohort in Mexico. *Environmental Health Perspectives* 123 (2):186-192. [https://doi.org/10.1289/ehp.1307476](https://doi.org/10.1289/ehp.1307476)


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