Mechanisms of Action for Arsenic in Cardiovascular Toxicity and Implications for Risk Assessment

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Introduction and Purpose

• Growing body of literature on potential association between inorganic arsenic (iAs) and cardiovascular disease (CVD)

• Purpose: Evaluate association between iAs and CVD, focusing on the strength of evidence for several proposed mechanisms of action (MoA) at lower iAs exposures
  ▪ In this assessment, "low" defined as <100 µg iAs/L water
Scope of Assessment

• Questions addressed:
  ▪ What are the proposed MoAs for iAs and CVD?
  ▪ Which MoA(s) is or are most plausible?
  ▪ What is the likelihood of initiation or exacerbation of CVD progression for US populations exposed to low iAs levels (<100 µg/L)?
  ▪ What other factors may modulate MoA(s) for iAs and atherosclerotic CVD in this population?
Literature Review

• Conducted two literature searches to capture animal and epidemiology literature
• Cross-checked materials with US EPA draft development materials for iAs IRIS assessment
• Reviewed >130 epidemiology abstracts for CVD and biomarkers; evaluated ~50 publications in detail
• Evaluated ~50 MoA studies
Background – Physiology of Cardiac Tissue

• Physiology of the arterial wall endothelium:
  1. Provides endothelial tissue barrier
  2. Produces antithrombotic molecules
  3. Prevents white blood cell adhesion/inflammation

• Function of vascular smooth muscle cells:
  1. Produces collagen and elastin to form extracellular matrix
  2. Produces inflammatory mediators (*e.g.*, interleukin-6, [IL-6])
  3. Regulated by growth promoters (*e.g.*, endothelin-1)
Atherosclerosis and Pathophysiology

- Atherosclerosis: Disease of arterial wall characterized by progressive inflammation, smooth muscle proliferation, the formation of plaques (atheromas), leading to narrowing of vasculature (e.g., epicardial coronary arteries)
Atherosclerosis Initiation and Progression

• Early changes in pathophysiologic development
  - Endothelial dysfunction → release of inflammatory molecules, expression of cell surface adhesion molecules, etc.
  - Increased endothelial permeability → lipoproteins deposit in the arterial wall, and plaque development/progression ensues
  - Recruitment of leukocytes, primary monocytes, and T lymphocytes → develop into foam cells
Atherosclerosis Progression

- Later steps in plaque formation, progression, and disruption
  - Foam cells recruit smooth muscle cells; other pro-inflammatory cytokines further plaque development
  - Collagen from smooth muscle cells stabilizes plaque and forms fibrous cap
  - Rupture of atherosclerotic plaque; possible myocardial infarction or stroke/cerebrovascular event
## Risk Factors for CVD

### Cardiovascular Disease Risk Factors

<table>
<thead>
<tr>
<th>Non-Modifiable Risk Factors</th>
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<tbody>
<tr>
<td>• Age</td>
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<td>• Sex</td>
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<td>• Family history and genetics</td>
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<td>• Race</td>
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<table>
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<tr>
<th>Modifiable Risk Factors</th>
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<tbody>
<tr>
<td>• Dyslipidemia</td>
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<tr>
<td>• Hypertension</td>
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<tr>
<td>• Type 2 diabetes</td>
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<tr>
<td>• Alcohol and tobacco smoking</td>
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<td>• Sedentary lifestyle</td>
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<td>• Obesity</td>
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Biomarkers of CVD

- CRP one of most sensitive and prognostic biomarkers
- Lp(a) and other biomarkers important in dyslipidemia and metabolic syndrome

### Selected Biomarkers of Cardiovascular Disease

<table>
<thead>
<tr>
<th>Inflammation</th>
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<tbody>
<tr>
<td>hsCRP</td>
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<tr>
<td>IL-6</td>
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<td>MPO</td>
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<td>Homocysteine</td>
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<th>Endothelial Dysfunction</th>
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<tr>
<td>VCAM-1</td>
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<td>sICAM-1</td>
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<th>Lipid Dysregulation</th>
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<tr>
<td>LDL-C</td>
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<td>HDL-C</td>
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<td>Lp(a)</td>
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Notes: HDL-C = high density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; IL-6 = interleukin-6; LDL-C = low-density lipoprotein cholesterol; LP(a) = lipoprotein (a); MPO = myeloperoxidase; sICAM-1 = soluble intracellular adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1
Mouse Models of CVD

• Apolipoprotein E Knockout (ApoE\(^{-/-}\))
  - Most commonly used
  - Readily develops atherosclerosis, even on low-fat diet

• Other Models
  - LDL receptor deficient (LDLr\(^{-/-}\))
    - Slow atherosclerosis development
  - ApoE*3-Leiden (E3L) transgenic
    - More sensitive to lipid-lowering drugs than other two models

• All models have differences in CV physiology relative to humans
iAs Metabolism

• ~95% of ingested soluble trivalent iAs compounds absorbed through gastrointestinal tract; from blood stream, taken up largely by cells in liver

• Undergo a series of methylation steps and are converted to easily excreted pentavalent organic metabolites MMA\textsuperscript{V} and DMA\textsuperscript{V}
  - Facilitated by enzyme arsenic methyl transferase (As3MT) \textit{via} transfer of a donor methyl group from S-adenosylmethionine (SAM)

• Methylation generates reactive trivalent intermediates MMA\textsuperscript{III} and DMA\textsuperscript{III}
Differences in Metabolism Across Species

• Rodent metabolism yields trimethyl arsenic, predominantly trimethylarsine oxide

• Rodents, particularly rats, retain arsenic in red blood cells

• Due to differences in toxicokinetics, higher doses typically required to induce similar target tissue concentrations in rodent models relative to humans
  - Concentrations of 100 µg/L water yield trivalent arsenical concentrations similar to those associated with 1-2 mg/L in rodents
Epidemiology of iAs and CVD

- High levels of iAs (most often, ≥300 µg/L) in drinking water associated with vascular disease
- Gaps and inconsistency in literature with regard to low to moderate exposures, particularly for US cohorts
- Exposure groupings limit interpretation and extrapolation
- Few studies consider the effect of dietary iAs
- Issues with interpretation of urinary arsenic measurements
## Selected Epidemiology Studies of iAs and CVD

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Type</th>
<th>Population/Study Size</th>
<th>Arsenic Level ((H_2O), \mu g/L)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. (2013)</td>
<td>Case-cohort</td>
<td>HEALS cohort (Bangladesh) N = 1,109</td>
<td>Mean: 96.4</td>
<td>Sig. ↑ risk of all CVD and HD &gt;108 (\mu g/L) but not below</td>
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<tr>
<td>Moon et al. (2013)</td>
<td>Prospective cohort</td>
<td>Strong Heart Study (Native Americans) N = 3,575</td>
<td>Not measured; urinary arsenic (iAs + methylated species) range: 0.1-183.4 (\mu g/g) creatinine</td>
<td>Sig. ↑ of CVD and CHD &gt;15.7 (\mu g/g) creatinine; sig. trend</td>
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<td>Moon et al. (2012)</td>
<td>Meta-analysis (31 studies)</td>
<td>Various countries</td>
<td>Individual studies with very high max (e.g., 930)</td>
<td>Sig. ↑ &gt;50 (\mu g/L)</td>
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<tr>
<td>Chen et al. (2011)</td>
<td>Prospective cohort</td>
<td>HEALS cohort (Bangladesh) n = 11,746</td>
<td>Range: 0.1-864.0 Mean: 99</td>
<td>Sig. ↑ risk of IHD and other heart disease at 148.1-864.0 (\mu g/L), but not below</td>
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<td>Lewis et al. (1999)</td>
<td>Retrospective cohort</td>
<td>Mormon group, Utah</td>
<td>Range of medians: 14-166 Max = 620</td>
<td>Sig. ↓ risk of all heart disease at mid- and high exposures; sig. ↑ risk in some groups for HHD</td>
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Notes: CHD = coronary heart disease; HD = heart disease; HEALS = Health Effects of Arsenic Longitudinal Study; HHD = hypertensive heart disease; IHD = ischemic heart disease; NR = not reported.
Modifying factors

- Nutrition
  - Folate intake; selenium?

- Genetic polymorphisms
  - As3MT

- Dietary iAs
  - Food and cooking water

Internal Dose & Susceptibility to iAs
Using MoA Data in Risk Assessment

• Seeks to define a set of "key events" between exposure and development of toxicity endpoint

• Qualitative and quantitative differences in dose-dependence of key events must be considered
MoA Framework for iAs and CVD

- Question for iAs may be how it interacts with and affects particular steps in the ongoing CVD process

Fig. 1. Schematic of the progression of clinical atherosclerosis and possible modification by inorganic arsenic exposure (adapted from Wu et al., 2014). Solid lines indicate established pathways; dotted lines indicate potential pathways. cIMT: carotid artery intima-media thickness, CRP: C-reactive protein, CVD: cardiovascular disease; Ox-LDL: oxidized low-density lipoproteins, PAI-1: plasminogen activator inhibitor-1, QTc: heart-rate corrected QT, QTD: QT dispersion, sVCAM-1: soluble vascular adhesion molecule 1, sICAM-1: soluble intercellular adhesion molecule 1, TDR: transmural dispersion of repolarization.
Proposed MoAs for iAs and CVD

• Immediate molecular targets
  ▪ Sulfhydryl binding to proteins, such as cysteine
  ▪ Affects wide variety of molecules; may bind to critical proteins for physiological functions
  ▪ Sufficient amounts would be needed, given indiscriminate nature and half-life of proteins
Proposed MoAs for iAs and CVD cont'd

• Endothelial dysfunction
  ▪ Caused by chronic inflammation, lipid peroxidation, or loss of vasodilatation
  ▪ Epidemiology studies show association with markers of inflammation at high levels of iAs or wide exposure ranges; association with oxidative stress less clearly established
  ▪ Animal studies show high iAs (orders of magnitude above arsenic MCL) may be associated with lesion formation and neovascularization, but inconsistent dose-response and issues with models
Proposed MoAs for iAs and CVD cont'd

- Hepatic dysfunction

Liver Disease → Systemic Inflammation → Atherogenesis?
In animals, risk of plaque formation increases with hepatic inflammation.

In humans, hepatic steatosis increases very low-density lipoprotein (vLDL) in humans and can exacerbate dyslipidemia.

- Adversity/clinical relevance in mechanistic animal studies unclear.
- Little evidence that liver is a target of toxicity at low iAs levels.
Plausibility of Proposed MoAs

• None of the available studies support direct causality
  ▪ Evidence does not support initiation or modification of CVD by low-level iAs

• Issues with physiological relevance (animal studies) and study design (animal and human studies)

• Most plausible scenario is that high levels of iAs interact with widely prevalent processes of CVD development (i.e., iAs does not act through a *de novo* chain of events)
Implications for Risk Assessment

• NRC recommended fitting flexible dose-response models to human studies
  ▪ Questions of exposure-response relationships given exposure measurement error in epidemiology studies
    • May bias toward linearization of apparent dose-response patterns
  ▪ Questions of generalizability given variation in genetic, nutritional, lifestyle, and other factors associated with CVD – how to adjust?
    ▪ Power to detect elevated risk is limited at low response rates
Additivity to Background and Homeostasis

• iAs' immediate molecular interactions are likely subject to feedback control
  ▪ Inappropriate signaling would advance to dysfunction only at exposures overwhelming the control system

This suggests that iAs' effects on CVD progression may operate via a threshold response

• Additivity to "background" CVD
  ▪ Concept is limited to a specific set of conditions (particularly, shared targets)
Conclusions

• Development of atherosclerosis result of complex pathophysiological process
• Many powerful risk factors for CVD (e.g., age, genotype, smoking, obesity)
• High iAs exposures associated with biomarkers of CVD and clinical disease
• Studies of intermediate markers show no convincing MoA at any exposure level/dose
• Few human studies have tested intermediate or apical endpoints at lower exposure levels
• Consideration of all evidence is consistent with a threshold dose-response relationship