Inorganic Arsenic Toxicity and Human Health

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Outline

1-Exposure
2-Toxicity
3-Treatment
4-Analytical methods
5-Summary
Concerns throughout the world with arsenic contamination of water and food.
Arsenic in drinking water

- Inorganic arsenic is carcinogenic: cancer of the bladder, lung, skin plus other internal organs.
- WHO recommendation: maximum contamination level 10µgAs/L drinking water.
- Bangladesh and West Bengal, India: Worst public health calamity in the last 50 yrs.
- SW Taiwan, Mongolia, China, Chile plus others.
Bangladesh and West Bengal disaster: Primary Cause: Ignorance of developed countries and their do-gooders! Interdisciplinary communications could have prevented it!

Environmental justice needed for children of the poor.
Other exposures

• Food (US dietary intake of inorganic As is 8 to 15µg/day)
• Soil, especially for children
• CCA (chromated-copper-arsenic) wood--children: play ground equipment
• Herbicides and pesticides
• Smelter emissions
• Mining
Exposure, (cont.)

- **Folk medicines**
  Many Chinese traditional drugs contain arsenic.

- **Arsenic in sea food** (arsenobetaine, arsenochocholine) is metabolically inert. Urine analysis can give false toxic exposure results.

- **Arsenic trioxide** for cancer chemotherapy
Results of chronic arsenic exposure

• Chronic exposure $\geq 300\mu g$ As/L in drinking water:
  – **Cancer**: skin, internal organs especially lungs and bladder.
  - Exposure of mice to arsenic during first half of pregnancy **leads** to hepatocellular carcinoma when the pups become adults.
  – Increased risk for vascular disturbances:
    e.g. blackfoot disease
Results of chronic exposure (cont.)
- Increased risk for diabetes
- Peripheral neuritis
- Risks associated with low concentrations in water??
- Association with decreased intelligence in children
- Some suggestions that children may metabolize arsenic differently than adults.
- Are children more susceptible?
Environmental justice for children of the poor
Acute arsenic exposure

• Criterion: urinary As conc. >175µg per 24 hrs

• Signs and symptoms: nausea, vomiting, diarrhea, hypotension, acute renal failure, tachycardia, malaise, seizure and coma.

• Use of hair analysis to obtain chronological history of exposure. (Hair growth ~ 1.5 cm per month).
Different arsenic species—Which is carcinogenic? One of them? All of them?
Biotransformation

Need to consider 6 not 1 arsenic compound for toxicity and carcinogenesis
Polymorphism and ethnic groups:
PNP
CYT 19
GSH transferase omega
As(V) As(III) MMA(V) MMA(III) DMA(V) DMA(III)

0 10 20 30 40 50 60 70 80

specific arsenic species, % of total urinary arsenic

Subject 44: E155del, E208K
Subject 47: E155del E208K, delGGC, A140D
La Virgen average
all subjects average

arsenic species
As(V) As(III) MMA(V) MMA(III) DMA(V) DMA(III)
Biotransformation

1. Arsenate reductase (purine nucleoside phosphorylase) converts arsenate to arsenite.
2. Arsenite/MMA\textsuperscript{III} methyltransferase converts arsenite to MMA\textsuperscript{V}.
3. MMA\textsuperscript{V} reductase (GST-omega) reduces MMA\textsuperscript{V} to dimethylarsinic acid (DMA\textsuperscript{V}).
4. Arsenite/MMA\textsuperscript{III} methyltransferase converts dimethylarsinic acid to methylarsonous acid (MMA\textsuperscript{III}).
5. DMA\textsuperscript{V} reductase (GST-omega) reduces DMA\textsuperscript{V} to dimethylarsinous acid (DMA\textsuperscript{III}).

Need to consider 6 not 1 As compound for toxicity and carcinogenesis.
MMA(V) Reductase Gene

GGCdel
S86C
A140D
E155del
G>T, A>T

Ex1
Ex2
Ex3
Ex4
Ex5
Ex6
E208K
A236V
G>A

kilobases
Treatment of arsenic toxicity

• REMOVE SOURCE OF EXPOSURE
• DMPS (2,3-dimercaptopropane1-sulfonate, Dimaval, Unithiol)
• DMSA (succimer, Chemet, meso-2,3-dimercaptosuccinic acid).
• BAL (BritishAnti-Lewsite, dimercaprol, 2,3-dimercaptopropane-1-hydroxyl)

When to use chelation?

Treatment of acute toxicity
After chronic exposure has ceased.
Analytical Methods

• Ion exchange
• Hydride generation-atomic absorption
• Hydride generation- atomic fluorescence
• HPLC-ICP-MS-----High performance liquid chromatography/inductively coupled plasma emission/mass spec.

HPLC-ICP-MS is the best. It is fast, accurate but expensive.
We have used these methods for studies in
Chile
Inner Mongolia
Southwest China
Romania
Mexico
Proteins and arsenic exposure

- Differential in-gel electrophoresis
- For comparing two samples
- Relative increased synthesis
- Relative decreased synthesis
Liver extract

Saline

Liver extract

Dye 1

Mixture 1

10 ul

10 ul

MIX SAMPLES 1 and 2
And place on gel

Dye 2

Mixture 2

Arsenite

Liver Extract

Dye 2
Proteins in Arsenite treated
Proteins in Saline treated

Proteins in Arsenite treated
Proteins in Saline treated
Proteins Not change

pH

Molecular weight
Acute arsenic exposure
Analysis of liver proteins after arsenite treatment in wild mice by DIGE System

**TREATED**: wild female mouse injected with **8 mg/kg of arsenite (sodium salt)** in **50 µL of saline** for 4 hours.

**CONTROL**: wild female mouse injected with **50 µL of saline solution** for 4 hours

Liver proteins were separated by Ettan DIGE System.
TREATED: *Wild* female mouse injected with 90 µg of Phenilarsine oxide in 50 µL of DMSO for 4 hours.

CONTROL: *Wild* female mouse injected with 50 µL of DMSO for 4 hours

Liver proteins were separated by Ettan DIGE System.
Summary

• Chronic exposure to about 300µg As/L in drinking water can cause cancer of the skin and internal organs, especially lungs and bladder.

• Children may be more susceptible to arsenic toxicity because of hand to mouth activity and developmental differences. Children are not small adults.

• Best treatment is to REMOVE SOURCE OF EXPOSURE. DMPS and DMSA available for chelation treatment.
Summary (cont.)

• Genetic factors e.g. polymorphisms are being investigated. Not many groups can do this. Robotization now available.

• HPLC/ICP/MS is the best analytical procedure available.

• Knowledge as to biotransformation of inorganic arsenic has increased. More is necessary to help risk assessment in humans.
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• NIEHS P30-ES06694
Thank you for your attention!
After PAO 4 hours treatment (90µg/20g mice) (4.5 mg/kg)

KO 19 proteins changed
    17 up-regulated
    2 down-regulated

W 3 proteins changed
    1 up-regulated
    2 down-regulated

After As(III) 4 hours treatment (380µg/20g mice) (19mg/kg = 24h LD50)

KO 13 proteins changed
    11 up-regulated
    2 down-regulated

W 7 proteins changed
    5 up-regulated
    2 down-regulated
Cytochrome B5: electron transport activity/fatty acid metabolism

Cytochrome C oxidase II: electron transport activity
*related to oncogenesis and iON carcinogenesis

Cytochrome C oxidase Vla-liver, mitochondrial

Respiratory problems in the mitochondria  Up-regulation of genes involved in the electron transport chain
Superoxide dismutase: antioxidant activity/neuroprotection

Peroxyredoxin 5: antioxidant activity/neuroprotection

GSTM1: antioxidant activity/inactivation of xenobiotics

Increase of free oxygen radicals after PAO and AsIII treatment (oxidative stress) → Up-regulation of antioxidant genes to protect the cell
60S ribosomal subunit: structural constituent of ribosomes

translational elongation

Peptidylpolyyl isomerase A: help in protein folding

Critical situation on the cell, necessary expression of protective genes

Up-regulation of genes involved in the transcription of genes and folding of proteins
Liver Protein Changes, DIGE method

Female Mice, 14 days, 100 mg Na arsenite/L water,
---CHRONIC EXPOSURE---
(These are fold changes in amounts not activities)

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetyl-CoA binding protein</td>
<td>+3.8 fold</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>+2.3</td>
</tr>
<tr>
<td>Cytochrome C oxidase Polypeptide VIb</td>
<td>+2.0</td>
</tr>
<tr>
<td>GST alpha</td>
<td>+1.5</td>
</tr>
<tr>
<td>Hbg beta chain</td>
<td>+1.5</td>
</tr>
<tr>
<td>Cytoskeletal keratin 18 (mouse)</td>
<td>+1.4</td>
</tr>
<tr>
<td>GST mu</td>
<td>+1.3</td>
</tr>
<tr>
<td>Carbonic anhydrase III</td>
<td>-1.4</td>
</tr>
<tr>
<td>SOD 1, soluble</td>
<td>-1.2</td>
</tr>
</tbody>
</table>
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Thank you for your attention!
Autistic Age 6 male vs Control Age 5 male
(blood plasma albumin and immunoglobulin were partially depleted)