Arsenicals, water treatment and water quality: Can we leave out one?

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ABSTRACT
Objective: Arsenic-associated human health complications are reported worldwide. Although inorganic arsenic has long been known to be toxic to humans, little is known about its metabolite, dimethylarsinic acid and its toxicity. We investigated the hepatic toxicity of dimethylarsinic acid and its interaction with iron and lipopolysaccharide in drinking water.
Materials and Methods: Rats were given drinking water with dimethylarsinic acid with or without iron for three weeks.
Results: Dimethylarsinic acid alone, iron alone, and dimethylarsinic acid-plus-iron treatment did not cause hepatic damage. A single dose of lipopolysaccharide increased hepatic damage in dimethylarsinic acid-plus-iron-treated rats.
Conclusion: We hypothesize that exposure to lipopolysaccharide increases hepatic damage in dimethylarsinic-acid-plus-iron-treated rats.

Key words: Dimethylarsinic acid, iron, lipopolysaccharide, hepatic damage, water treatment

INTRODUCTION

Arsenic is associated with an increased risk of cancer in the skin, lungs, liver, kidneys, and urinary bladder of humans[1]. In the liver, and possibly other organs, inorganic arsenic is metabolized to methylated compounds[monomethylarsinic acid (MMA) and dimethylarsinic acid (DMA)]. Although methylation is believed to decrease the biological activity of arsenic[2], this has too been questioned[3]. Data from free radical, biochemical, and carcinogenic studies of DMA suggest that the methylation of arsenic may instead be a pathway of toxification[4-8].

Concern about human exposure to DMA has focused on its use as an herbicide or on its residual presence in food products. However, there are other significant sources of exposure to this agent[9]. Inorganic arsenic ingested as a contaminant of drinking water and in some foodsis biologically methylated[10-12], yielding MMA and DMA. Another significant source is seafood that contains a high concentration of DMA[13,14]. The toxicity of inorganic arsenicals, arsenite, and arsenate has been extensively studied; however, the toxic manifestations of methylated arsenic metabolites have not been well characterized.

The risk of outbreaks of waterborne diseases increases where standards of water, sanitation and personal hygiene are low. In 2009, it was estimated that diarrhoea due to unsafe water and a lack of basic sanitation contributed to the death of 1.5 million children aged less than five years each year[15]. Contaminated drinkingwater is a frequent cause of diseases such as cholera, typhoid, viral hepatitis A and dysentery. Water may be contaminated with naturally occurring inorganic elements such as arsenic, radon or fluoride. Human activity may also cause water to become contaminated with substances such as lead, nitrates and pesticides[16].

The risk for developing a human disease derived from environmental exposure is not based solely on the environmental exposure, but is modified

by mitigating conditions, such as other environmental or genetic factors[17]. Iron may be important in arsenic-associated toxicity[18,19]. DMA releases iron from ferritin in vitro, which initiates the generation of hydrogen peroxide[6]. In addition, sub-hepatotoxic arsenic exposure enhances lipopolysaccharide (LPS)-induced liver damage[17]. Many arsenic removal plants in arsenic-contaminated areas use filtration technologies that contain iron. Whether LPS modifies the risk of DMA-plus-ironin the liver damage has not been determined. This study examined the effects of sub-chronic exposure of DMA with/without iron and LPS on DMA-plus-iron in the hepatic toxicity in rats.

MATERIALS AND METHODS

Chemicals

Dimethylarsinic acid (DMA), ferrous sulphate, and lipopolysaccharide (LPS; derived from *Escherichia coli* serotype 055:B5) were purchased from Sigma-Aldrich (St. Louis, MO).

Animals

Ten-week-old male Wistar rats (250 to 300 g) purchased from our Institutional Laboratory Animal Center were used in this study. They were given pellet feed (Richmond Standard; PMI Feeds, Inc., St. Louis, MO) and water. They had a 12-h light/dark cycle and central air conditioning (25°C, 70% humidity) throughout the experimental period. The animal care and experimental protocols were in accordance with nationally approved guidelines.

Experimental Design

Experiment 1: DMA dose response study.

Forty-eight male rats were divided into six groups of eight. Group I, the healthy controls (HC), was given only normal drinking water; groups II to VI were given DMA in drinking water (10, 30, 100, 300, and 1000 ppm) for three weeks. Aspartate transaminase (AST) and alanine transaminase (ALT) levels were assessed in serum after three weeks of treatment.

Experiment 2: Effect of DMA and iron exposure in rats. Forty-eight rats were divided into eight groups of six. Group I (HC) was given only normal drinking water. Group II (DMA) was given only DMA (1000 ppm) in drinking water. Groups III to V were given different doses (100, 200, and 300 ppm) of iron[as ferrous sulphate(Fe in the figure)] in drinking water, respectively. Groups VI to VIII were given DMA (1000 ppm) and different doses of Fe (100, 200, and 300 ppm). After 3 weeks of treatment, AST and ALT levels were assessed in serum.

Experiment 3: Effects of LPS on rats pre-treated with DMA and iron.

Forty-eight rats were used for two sets of separate experiments. The first 24 rats were randomly divided into four groups of six. Group I was given only normal drinking water; Group II was given normal drinking water and, on day 7, a single intraperitoneal (i.p.) injection of LPS (0.01 mg/kg), and 12 h later they were killed; Group III was given DMA (1000 ppm) and iron (300 ppm) in drinking water; Group IV was given the same doses of DMA and iron as Group III plus, on day 7, a single injection of LPS (0.01 mg/kg; i.p.) and 12 h later they were killed. After 7 days, all these rats were killed. The second 24 rats were randomly divided into four groups of six and given the same treatment as the first 24. After 14 days, AST and ALT levels were assessed in serum.

Collecting blood and biochemical analysis

Blood was collected in serum separation tubes from a femoral vein via venipuncture while the rats were under mild ether anesthesia. The tubes were left at room temperature for 30 min to clot and then centrifuged at 1000 ×*g* at 4°C for 10 min. The serum AST and ALT levels were determined using a blood biochemical analyzer (DRI-CHEM 3500s; Fujifilm, Kanagawa, Japan).

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) and then with Tukey's multiple comparison tests to evaluate the significance between the treatment groups. Values are means ± standard deviation. Statistical significance was set at $P < 0.05$.

RESULTS

Effects of DMA treatment on rat liver

To investigate the effects of rat livers exposed to DMA, we determined AST and ALT levels in rat serum. Neither AST (range: 69.50±13.21-82.63 ± 3.78 U/L) nor ALT (48.50 ± 15.36-58.13 ± 4.42 U/L) levels were significantly different (Table 1).

Table 1.Effects on rat liver of DMA treatment.*

DMA (ppm)	AST (U/L)	ALT (U/L)
Group I: 0	78.43 ± 6.21	52.57 ± 6.05
Group II: 10	82.63 ± 3.78	58.13 ± 4.42
Group III: 30	77.75 ± 11.70	49.75 ± 11.56
Group IV: 100	82.38 ± 15.66	49.38 ± 5.60
Group V: 300	78.50 ± 18.03	51.25 ± 13.22
Group VI: 1000	69.50 ± 13.21	48.50 ± 15.36

*Forty-eight rats were divided into six groups of eight. Group I, the controls (HC), was given drinking water without DMA; groups II to VI were given DMA in drinking water (10, 30, 100, 300, and 1000 ppm) for three weeks. Aspartate transaminase (AST) and alanine transaminase (ALT) levels were assessed in serum after 3 weeks of treatment. Data are means ± standard deviation (P< 0.05; one-way ANOVA and then Tukey's multiple comparison tests).

Effects of Fe on rat liver exposed to DMA

To determine the effects of Fe on DMA-exposed rat livers, we measured AST and ALT levels in rat serum. Neither AST (range: 72.25 ± 11.11-83.67 ± 8.08 U/L) nor ALT (41.25 ± 8.38-51.75 ± 6.39 U/L) levels were significantly different in rats co-treated with DMA-plus-Fe (Table 2).

Effects on rat liver co-treated with DMA, Fe, and LPS

To investigate the effects of LPS on DMA- and Fe-exposed rat livers, we measured serum AST and ALT levels. LPS treatment on 7 and 14 days significantly (P< 0.05) increased the AST and ALT levels in DMA-plus-iron-exposed rat livers (Figure 1). Neither, DMA plus LPS nor Fe plus LPS treatments altered the serum AST and ALT levels.

Table 2.Effects of iron on DMA-exposed rats.*

Treatment (ppm)	AST (U/L)	ALT (U/L)
Group I: HC	72.25 ± 11.11	51.75 ± 6.39
Group II: DMA (1000)	81.00 ± 8.48	45.25 ± 8.48
Group III: Fe (100)	77.50 ± 8.85	41.25 ± 8.38
Group IV: Fe (200)	78.00 ± 4.96	42.75 ± 2.98
Group V: Fe (300)	77.15 ± 4.97	43.50 ± 9.74
Group VI: DMA (1000) + Fe (100)	82.15 ± 9.91	50.50 ± 9.19
Group VII: DMA (1000) + Fe (200)	83.67 ± 8.08	50.67 ± 7.57
Group VIII: DMA (1000) + Fe (300)	80.33 ± 6.11	48.18 ± 8.89

*Forty-eight rats were divided into eight groups of six. Group I (HC) was given only normal drinking water. Group II (DMA) was given only DMA (1000 ppm) in drinking water. Groups III to V (Fe) were given different doses (100, 200, and 300 ppm) of iron (as ferrous sulphate [Fe in the figure]) in drinking water, respectively. Groups VI to VIII were given DMA (1000 ppm) and different doses of Fe (100, 200, and 300 ppm). After 3 weeks of treatment, AST and ALT levels were assessed in serum. Data are means ± standard deviation (P< 0.05; one-way ANOVA and then Tukey's multiple comparison tests).

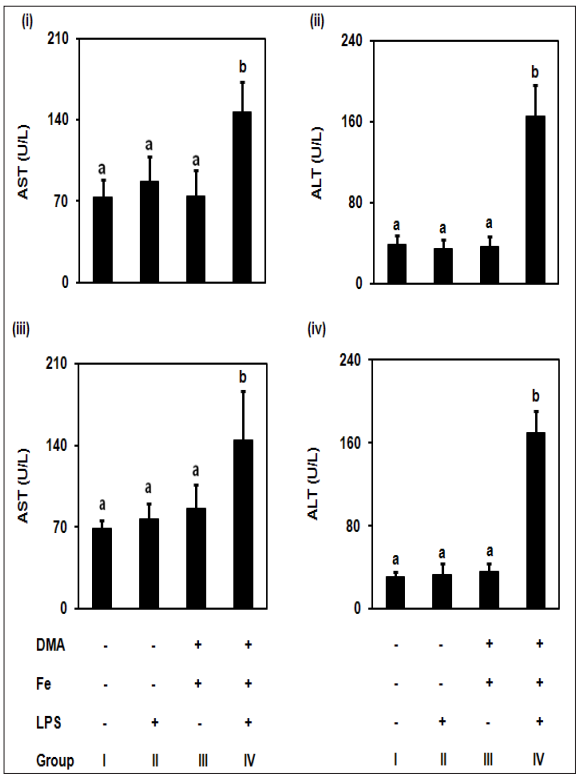

Figure 1.Effect on rat liver of co-treatment with DMA, iron, and LPS. Two sets of twenty-four rats (Study I and Study II) were divided into four groups. Study I: Group I, received normal drinking water. Group II, received LPS [0.01 mg/kg; intraperitoneally (i.p.)]. Group III received DMA and iron (1000 and 300 ppm, respectively) in drinking water. Group IV first received DMA and iron and then a single injection of LPS (0.01 mg/kg; i.p.). In Groups II and IV, LPS was given just 12 h before they were killed with Groups I and III on day 7, after which their serum aspartate transaminase [AST (i)] and alanine transaminase [ALT (ii)] level were measured. Study II, the same conditions as in Study I, except that Study II continued for 14 days. After 14 days, the rats were killed and their serum AST (iii) and ALT (iv) levels were measured. Data are means ± standard deviation. Different letters (a, b) indicate a significant difference between groups (P< 0.05; one-way ANOVA and then Tukey's multiple comparison tests)

DISCUSSION

Arsenic contamination of drinking water is reported in many parts of the world. Metal salts have been used to remove arsenic since at least 1934. The most commonly used metal salts, aluminum salts such as alum, and ferric salts such as ferric chloride, ferric hydroxide, and ferric sulfate[20,21] provide excellent arsenic removal. Laboratories report over 99% removal under optimal conditions, with residual arsenic concentrations of less than 1 µg/l[22]. Zero-valent iron filings can be used either in situ or ex situ to reduce arsenate and produce ferrous iron. However, treated water is very high in ferrous iron. Therefore, it must undergo iron removal treatment before it is distributed and drunk[23]. Water treatment systems such as iron-based precipitation to remove arsenic from drinking water is widely used[24-26]. However, acute exposure of rats to sodium arsenite-plus-iron synergistically induces hepatic damage mediated through oxidative stress19. As arsenic removal plants in arsenic contaminated areas use filtration technologies that contain iron, precautions must be taken to reduce the risk of ionized iron interacting with acute exposure of arsenic[19]. In contrast, sub-chronic exposures of DMA, Fe, and DMA-plus-Fe in drinking water did not induce hepatic injury in rats. We suggest that inorganic arsenic metabolized to methylated compounds do not interact with iron to induce hepatic damage.

Co-treated with DMA, Fe, and LPS significantly increased hepatic injury in rats. Waterborne diseases arise from the contamination of water, either by pathogenic bacteria or protozoa or by chemical substances. These agents are directly transmitted to people when the water is used for drinking, preparing food, recreation or other domestic purposes[16]. Although the high dose of LPS, the outer membrane of bacteria induces liver damage[17], rats treated with one single non-effective dose of LPS after subchronic exposure of DMA-plus-Fe either on day 7 or day 14 experienced a significant increase in hepatic damage. We hypothesize that DMA-plus-Fe interacts with LPS to increase hepatic injury in rats.

CONCLUSION

Drinking water, after food, represents a secondary source of inorganic arsenic in human system. Long term exposition to arsenic may cause a wide range of health effects. New guidelines have been recommended to remove arsenic from drinking water. Further, various arsenic removal technologies that include conventional adsorbents and different chemicals have been used. It is possible

that the contamination of water by bacteria resulted in LPS interaction with iron-based precipitation system to remove arsenic from drinking water and causes hepatic injury. Our study indicates an important public health issue: the arsenic removal system may cause liver damage via arsenic metabolites as well as poor water quality. However, further studies are needed to confirm this.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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