Clinical Chemistry 45:2
301–306 (1999)

Case Report

Arsenic Speciation in Human Organs following Fatal Arsenic Trioxide Poisoning—A Case Report

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The aim of this investigation was to study the distribution of arsenic species in human organs following fatal acute intoxication by arsenic trioxide. The collected autopsy samples of most organs were ground and dried, and the total arsenic was measured by electrothermal atomic absorption spectrometry (ETAAS). The arsenic species—inorganic arsenic, in the form of arsenite [As(III)] and arsenate [As(V)], and its metabolites [monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)]—were quantified by ETAAS after extraction with methanol/water (1:1, by volume) and separation by HPLC. The results indicate that after acute intoxication, the liver and kidneys show the highest concentrations of total arsenic and that the total concentration in blood is 7- to 350-fold less concentrated than in organs. In all organs, As(III) is the predominant species, and MMA is more concentrated than DMA. MMA and DMA are more prevalent in lipidic organs (49% of total arsenic) compared with other organs (25% of total arsenic). As(V) was found in small quantities in the liver, kidneys, and blood.

We present here the first study to our knowledge of the distribution of arsenic species in most organs of a human after fatal intoxication by arsenic trioxide. The 28-year-old man died 3 days after committing suicide by oral absorption of a massive dose (~8 g) of arsenic trioxide. The victim found arsenic trioxide in his grandmother's pharmacy. The clinical picture was characterized by hepatonephritis, cardiomyopathy, and finally, a fatal rhabdomyolysis associated to hemolysis. During the autopsy, specimens of most of organs were taken, deep-frozen, and stored before analysis.

The arsenicals have been well known as poisonous agents since antiquity and have been used frequently in homicides and suicides. Lethal acute intoxication by arsenic has become rare, although arsenic is still sometimes used for suicide.

The acute toxicity of arsenic has been shown to vary according to the chemical species involved. The 50% lethal doses for oral administration to mice are as follows: 3 mg/kg for arsine; 14 mg/kg for arsenite [As(III)]; 20 mg/kg for arsenate [As(V)]; 700-1800 mg/kg for monomethylarsonic acid (MMA);[4] 700-2600 mg/kg for dimethylarsinic acid (DMA); and >10 000 mg/kg for arsenobetaine and arsenocholine *(1)*. Therefore, the inorganic species of As(III) and As(V) are more toxic than their methylated forms MMA and DMA. Arsenobetaine and arsenocholine are nontoxic and are eliminated rapidly in the urine after absorption. The lethal dose of inorganic arsenic is 100–200 mg of arsenic trioxide for an adult individual, although people have survived larger doses *(2, 3)*.

After its absorption, inorganic arsenic is partly methylated into MMA and DMA, which are excreted largely in the urine. One fraction of the remaining arsenic is eliminated unchanged in the urine; the second fraction is retained in the organism *(2, 4, 5)*.

In healthy humans exposed to trace amounts of arsenic in their environment, the highest concentrations of arsenic are found in tissues rich in sulfhydryl groups, such as the skin, hair, and nails *(2)*. Little is known about the total arsenic concentrations in the organs of unexposed individuals; the concentrations in blood and urine are 2.5 μg/L and 10–50 μg/L, respectively *(2)*.

When larger doses of arsenic are ingested, the tissue distribution appears to change. Many studies have been performed in humans after fatal arsenic poisoning *(6–8)*. The results of these studies showed widespread distribution of arsenic in all organs; the highest concentrations

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Received August 12, 1998; revision accepted November 13, 1998.

[4] Nonstandard abbreviations: MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AAS, atomic absorption spectrometry; and TBAH, tetrabutylammonium hydroxide.

were in the liver and kidneys, which had, respectively, 10- and 3-fold higher concentrations than in the other organs (brain, cerebellum, lung, heart, pancreas, spleen, muscle, and skin). Although the distribution of total arsenic in tissues has been well studied in humans *(6–8)*, few studies on the speciation of arsenic in animals have been reported in the literature, most of which focused on speciation in hepatic tissues following experimental acute intoxication *(9–12)*.

Here, we report a first case of arsenic speciation in human organs. The distribution of the arsenic species [As(III) and its metabolites] in each organ was studied, and the results were compared with those in the literature. Some of our results confirm known toxicological and metabolic mechanisms of inorganic arsenic in the human organism; others have led us to propose new mechanisms.

Arsenic speciation was performed using HPLC in the ion-pairing mode followed by arsenic determination by atomic absorption spectrometry (AAS).

Materials and Methods

REAGENTS

Stock solutions of 1 g/L arsenite [As(III)], arsenate [As(V)], MMA, and DMA salts were prepared by dissolving the corresponding powders in doubly deionized water: arsenic trioxde, As_2O_3 (Sigma Chemical); sodium arsenate, $Na_2HAsO_4 \cdot 7\ H_2O$ (Sigma); sodium monomethylarsonate, $CH_3AsO(ONa)_2 \cdot 6\ H_2O$ (Carlo Erba); and sodium dimethylarsonate (cacodylate), $(CH_3)_2AsO(ONa)$. All concentrated solutions were stored at 4 °C in the dark and used for the daily preparation of diluted calibrator solutions.

Mineralization was performed using nitric acid (Merck); deionized water and methanol (Carlo Erba) were used for arsenic extraction.

The mobile phase was prepared using deionized water; 400 mL/L tetrabutylammonium hydroxide (TBAH) in water (Fluka) was used as a counter anion, and orthophosphoric acid (normapur grade; Prolabo) was used to adjust the pH. Before use, mobile phase was filtered through a 0.2 μm cellulose acetate membrane (Millipore).

For graphite furnace AAS, 0.5 mL/L Triton X-100 (Merck) was used for sample dilution and 10 g/L $Ni(NO_3)_2 \cdot 6\ H_2O$ (Merck) was used as the matrix modifier. A 50 μg/L aqueous arsenic solution, prepared daily from a 1 g/L arsenic standard solution (Spex), was used to calibrate the AAS instrument.

We used a certified reference material: DORM-2 (dogfish muscle) from the National Research Council of Canada.

INSTRUMENTATION

For separation of the arsenic compounds by HPLC, the chromatographic system used included an isocratic pump (Shimadzu; model LC-6A) and a Rheodyne valve fitted with a 20-μL sample loop.

Separation was performed in ion-pair mode on a reversed-phase silica-based octadecyl (C_{18}) column resistant at pH 2–12 pH (SMT OD-5-100, 250 × 4.6 mm i.d.).

For the chromatographic optimization step, we used an ultraviolet detector (Shimadzu; model SPD-6A).

The determination of arsenic species in organ samples was performed using a SpectrAA Zeeman 220 (Varian) equipped with a pyrolytic graphite-coated graphite furnace, and a Zeeman-effect background corrector.

PROCEDURES

Specimens of organs (brain, cerebellum, heart, lungs, liver, kidneys, pancreas, spleen, muscles, and skin) were collected, cut into small pieces, and ground in a mixer (POLYTRON®). Portions of the ground organs were divided in two aliquots, one for total arsenic determination and the other for speciation. The accurately weighed organ samples were heated for 6 h in an oven at 60 °C and completely dried for 3 h at 105 °C. The dried samples were weighed and again put in the oven until the weight was constant.

Total arsenic concentrations were quantified by AAS of dried powder samples of the organs after mineralization by nitric acid. The mean (± SD) total arsenic content determined for the reference material was 17.05 ± 1.6 μg/g (certified value, 18 ± 1.1 μg/g).

We extracted the arsenic species from the second powdered sample of each organ (400–500 mg dried weight) by adding 2 mL of 500 mL/L water–500 mL/L methanol *(13, 14)* and shaking the mixture for 20 min. The solution was centrifuged at 1950*g* and the remaining powder was extracted four more times, using the same procedure. The five extracts thus obtained were collected and evaporated. To obtain a concentrated arsenic solution from each residue (>800 μg/L), an appropriate volume of deionized water was added. This volume was determined after measurement of the total arsenic in the mineralizates. The solutions were shaken 20 min, centrifuged, and filtered through a 0.2 μm cellulose acetate membrane (Millipore).

For the chromatographic separation of arsenic species, we adapted our previously published method *(15)* for the separation of six arsenic species [As(III), As(V), DMA, MMA, arsenobetaine, and arsenocholine] in the ion-pairing mode. Optimization of the pH and counter ion (TBAH) concentration in the mobile phase was achieved for good separation of As(III), As(V), DMA, and MMA.

The study of the capacity factors of As(III), DMA, MMA, and As(V) as a function of the TBAH concentrations at neutral pH showed that the separation between DMA and the MMA is better at lower TBAH concentrations. The chosen concentration was 3 mmol/L. At this concentration, the influence of pH on the separation was studied, and pH 8 was selected as the optimal pH.

The selected working mobile phase contained 3 mmol/L TBAH in aqueous solution at pH 8. The flow rate was 0.8 mL/min. The retention times for the arsenic species, determined in a previous step by use of concen-

Table 1. Total concentrations of arsenic in organs and blood.

Organ	Dry weight, μg/g
Liver	147
Kidneys	26.6
Muscle	12.3
Heart	11.75
Spleen	11.72
Pancreas	11.2
Lungs	11.13
Cerebellum	10.95
Brain	8.33
Skin	2.9
Hemolyzed blood	0.422

Table 2. Extraction yield of total arsenic species from organs.

Organ	Yield, %
Brain	98
Cerebellum	96
Liver	95
Spleen	95
Lungs	85
Pancreas	85
Muscle	75
Heart	73
Kidneys	73
Skin	71

trated calibrators detectable by ultraviolet absorption, were as follows: As(III), 3.60 min; DMA, 6.60 min; MMA, 8.50 min; and As(V), 16 min. In a second step, the organ extracts were filtered through a 0.2 μm Millipore membrane and injected into the column. Each fraction of As(III), DMA, MMA, and As(V) was collected and concentrated if necessary by evaporation at 40 °C. Each arsenic species in each separated fraction was determined by AAS. The chromatogram of a liver extract, with the arsenic species identified for the corresponding fractions, is shown in Fig. 1. Fractions (0.8 mL) were collected at 1-min intervals.

Results

TOTAL ARSENIC CONCENTRATIONS

The total arsenic concentrations in organs (relative to dry weight) are presented in Table 1. They show that arsenic is ubiquitous in all organs and that the liver and kidneys contain the highest concentrations (147μg/g in the liver and 26.6 μg/g in the kidneys). Other organs contain lower, almost identical concentrations (8–12 μg/g). The total arsenic concentration is lowest in the skin (2.9 μg/g).

EXTRACTION YIELDS

The extraction efficiency depends on each organ. The results presented in Table 2 show that the percentage of extraction by the methanol/water mixture (calculated with mineralization as the reference method) is between 71% and 98%.

DETERMINATION OF CONCENTRATIONS OF ARSENIC SPECIES IN ORGANS

The concentrations of the arsenic species in different organs are presented in Table 3. In all tissues, As(III) was the main species found, comprising 75–85% of the total arsenic in most organs except in the cerebellum, brain, and skin (47%, 53%, and 56%, respectively). The proportions of the metabolites DMA and MMA are relatively small, and As(V) concentrations are lower than the limit of quantification except in the liver and kidneys.

Discussion

TOTAL ARSENIC

The results of the total arsenic determination in tissues (Table 1) show that the arsenic tissular distribution is ubiquitous and that the highest concentration is found in

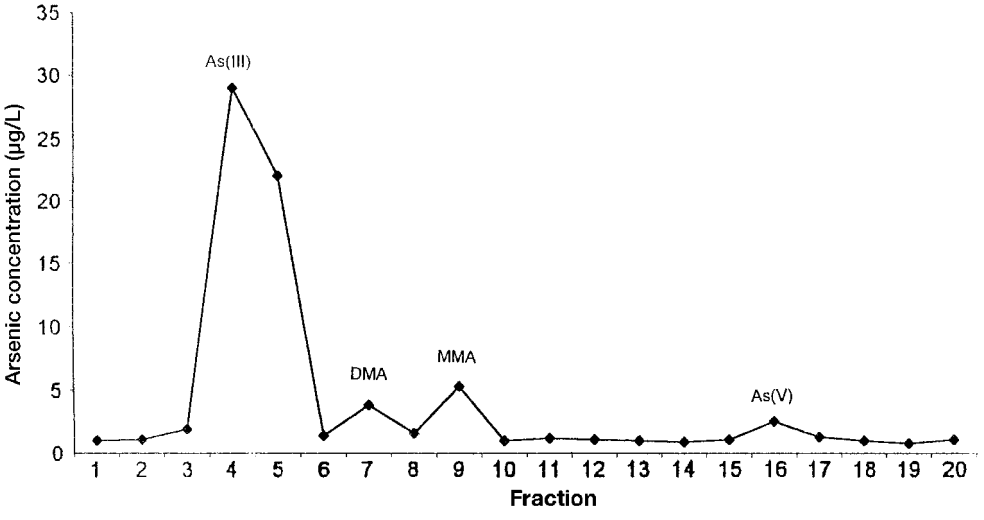

Fig. 1. Representative chromatogram of four arsenic species extracted from liver.

Separation conditions as described in *Materials and Methods*.

Table 3. Concentrations of arsenic species in organs and blood.

Organ	Concentration, μg/g dry weight					As(III)/ [Mb + As(V)]	MMA/DMA
	As(III)	DMA	MMA	As(V)	Mb[a] + As(V)		
Liver	122.00 (83%)[b]	5.91 (4%)[b]	14.7 (10%)[b]	2.94 (2%)[b]	23.55 (16%)[b]	5	2.5
Kidneys	19.95 (75%)	1.60 (6%)	4.52 (17%)	0.53 (2%)	6.65 (25%)	3	2.8
Muscle	9.17 (75%)	0.73 (6%)	1.96 (16%)	<LOQ[c]	2.69 (22%)	3	2.7
Heart	9.05 (77%)	0.64 (5%)	1.61 (14%)	<LOQ	2.25 (19%)	4	2.5
Spleen	9.50 (81%)	0.59 (5%)	1.65 (13%)	<LOQ	2.24 (18%)	4	2.8
Pancreas	9.18 (82%)	0.45(4%)	1.34 (10%)	<LOQ	1.79 (14%)	5	3
Lung	9.46 (85%)	0.45 (4%)	1.11 (10%)	<LOQ	1.56 (14%)	6	2.4
Cerebellum	5.15 (47%)	1.60 (19%)	3.76 (30%)	<LOQ	5.36 (49%)	1	2.4
Brain	4.41 (53%)	1.10 (18%)	2.65 (27%)	<LOQ	3.75 (45%)	1	2.4
Skin	1.62 (56%)	0.34 (15%)	0.91 (28%)	<LOQ	1.25 (43%)	1	2.7
Hemolyzed blood	0.224 (53%)	0.0539 (13%)	0.135 (32%)	0.009 (2%)	0.198 (47%)	1	2.5

[a] Mb, DMA + MMA.
[b] Percentage of total arsenic.
[c] LOQ, limit of quantification.

the liver, followed by the kidneys. These findings confirm those reported in several studies of fatal acute intoxication by inorganic arsenic in humans *(6, 8)* and animals *(9–12)*.

The concentration of arsenic in the liver and kidneys can be explained by the role of these organs in the detoxification (the liver is the site of inorganic arsenic methylation) and elimination of arsenic. It can also be noted the high concentrations found in muscle and heart (third and fourth highest arsenic concentrations after the liver and kidneys) tissue, which probably explains the fatal rhabdomyolysis observed with heart failure.

When we compare the concentration of total arsenic in all organs with the concentration in the blood (Table 1), we observe that the concentrations in the organs are ~7- to 350-fold higher than in blood. The blood sample studied was collected in the few hours before death during the period of intense hemolysis.

The liver is the major target organ of inorganic arsenic, which is explained by the affinity of As(III) toward vicinal dithiol in hepatic cytosolic proteins. The concentration of total arsenic found in this organ in our reported case was 5- to 50-fold higher than in most other organs (Table 1). Arsenic is distributed almost equally throughout most of the other organs (8–12 μg).

The binding of arsenic in target organs was postulated as a first step of arsenic detoxification. Nevertheless, this binding is also responsible for the major intoxication symptoms: hepatic and renal failure and cardiovascular and neurological effects. Marafante et al. *(9)* performed an in vivo study in rabbits to determine the arsenic distribution in tissues. The results showed that 5 h after arsenite administration, 11.6%, 1.4%, and 1.7% of the dose was distributed in the liver, kidneys, and lung, respectively, most of which was bound to proteins. This binding occurred within 1 h of either arsenite or arsenate administration *(16, 17)*. The binding of arsenic to tissue proteins

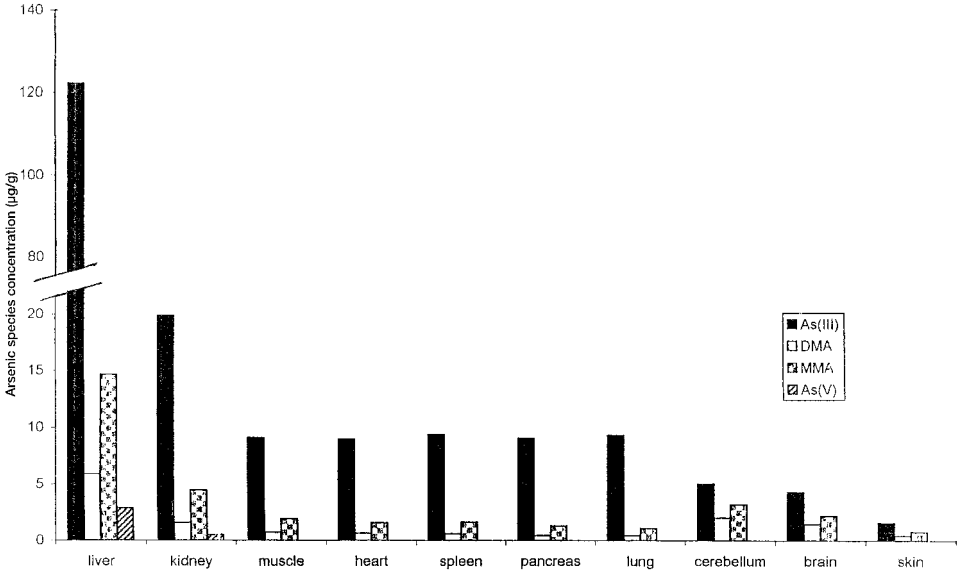

Fig. 2. Concentrations of arsenic species in organs (μg/g dry weight).

has been proposed as an additional step or perhaps the first step in the detoxification of inorganic arsenic before methylation *(18, 19)*.

ARSENIC SPECIATION

Studies in the literature have demonstrated that, in animals, As(III) is the major arsenic species present in tissues after experimental intoxication with As(III) or As(V). These results implicate As(III) as the main form of arsenic bound to tissues, compared with As(V) and the methylated metabolites. In addition, these studies showed that, in acute intoxication, arsenic cannot be completely detoxified by the organism and binds to tissues. As(III) is the main form of arsenic bound to hepatic tissue, compared with As(V) and DMA *(10, 16)*. Bogdan et al. *(10)* reported that when hepatic cytosolic proteins were incubated with inorganic arsenic, the amount of As(III) bound to these proteins was 13-fold greater than the amount of As(V) bound. In animals given As(III), the majority of absorbed As(III) was found in the liver, notably in the cytosol (50–65%) *(20)*. This difference in protein binding might be attributable to the affinity of As(III) for sulfhydryl groups, especially vicinal dithiol *(10)*.

These observations made in animal experiments appear to be confirmed in humans by our results (Fig. 2): we found As(III) to be three- to sixfold more concentrated than other species (Table 3, column 7). We also observed for the first time a lower ratio of As(III) to other arsenic species in lipid-rich organs (cerebellum and brain). This difference can be explained by the increased affinity of the metabolites toward lipids compared with As(III). Skin appears to be rich in the methylated metabolites, although if has the lowest total arsenic content among all tissues. In acute intoxication, compared with chronic intoxication, skin concentrations are low *(2, 6–8)*. The increased concentration of arsenic metabolites in skin compared with other organs remains unexplained. The ratio also equals unity in blood.

Our speciation results show the presence of four arsenic species: As(III), which is absorbed but not metabolized, MMA, DMA , and As(V).

These findings are in concordance with the knowledge of arsenic metabolism in humans. Indeed, the methylation pathway involves the formation of a monomethylated metabolite of As(III), which is either rapidly methylated again into a dimethylated derivative or is spontaneously oxidized into the As(V) form MMA *(12)*. The first methylation reaction is catalyzed by arsenite methyltransferase. The transfer of the second methyl group is catalyzed by another enzyme, MMA methyltransferase *(20)*. The latter enzyme is sensitive to inhibition by As(III) *(11, 12)*. This characteristic explains the increased concentrations of MMA in biological media after acute intoxication *(3, 12, 21)*. As(V) is reduced to As(III) by arsenate reductase before its methylation. In addition, a small percentage of total arsenic is found in blood and urine as the As(V) form and was shown to originate from in vivo oxidation of absorbed As(III); the mechanism of this oxidation, however, is unknown *(2, 21)*. When viewed collectively, studies of the transformations of ingested inorganic species in humans and mammals *(2, 11, 12, 21, 22)* lead to the postulation of a general mechanistic sequence: As(V) $\leftrightarrows$ As(III) $\rightarrow$ MMA $\rightarrow$ DMA.

The methylated metabolites (MMA and DMA) show a very weak affinity for tissues; therefore, their elimination in bile, blood, and urine is rapid *(2, 10, 23)*. Many studies have shown that MMA is the major metabolite in acute intoxication, whereas DMA is the major metabolite in chronic exposition *(2, 23–25)*.

The MMA/DMA ratios in the tissues in our study (Table 3, column 8) indicate that MMA is 2.5- to 3-fold more concentrated than DMA, which is characteristic of acute intoxication *(3, 12, 21)*. The much higher concentrations of MMA compared with DMA can be explained by the modification of the mechanism of methylation in acute intoxication. In this case, MMA methyltransferase is inhibited by the high concentration of As(III).

The amounts of the metabolites (Table 3, column 6) are lower than As(III); these species are eliminated rapidly in the bile and blood after methylation because of their weak affinity for tissues.

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