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Tissue distribution of arsenic species in rabbits after single and multiple parenteral administration of arsenic trioxide: tissue accumulation and the reversibility after washout are tissue-selective

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Abstract Parenteral administration of arsenic trioxide has recently been recognized as an effective antineoplastic therapy, especially for the treatment of acute promyelocytic leukemia. Its efficacy and toxicity are concentration-dependent and are related to the fractions of different arsenic species and the degree of methylation. In this study, arsenic trioxide was given parenterally to rabbits as a single dose or as a daily dose (0.2, 0.6, and 1.5 mg/kg) for 30 days. The blood and organ concentrations of the arsenic species, including As(III), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA), were studied on day 1 (single-dose study), day 30 (multiple dosing study), and day 60 (reversibility study). As(III) was the major detectable arsenic species in the blood. The pharmacokinetic parameters (total clearance, area under the curve, etc.) for As(III) indicated a limit for the capacity to eliminate As(III) at the dose of 1.5 mg/kg, and were quite the same after a single dose or chronic multiple dosing. In tissues, DMA was found to be the major metabolite and the concentrations of DMA, As(III), and MMA in general increased with the dose, with the increase most significant at a dose of 1.5 mg/kg. However, normalized tissue distribution of As(III) in the kidney on day 1, but not on day 30, was nonlinear. Along with decreased levels of As(III) and increased levels of DMA, an inducible capacity for methylating As(III) to DMA after chronic dosing in kidney was suggested. The tissue concentration of DMA was highest in lung and liver, and the normalized tissue distributions in liver on day 30 were nonlinear, suggesting a limit in eliminating DMA after a chronic high load of As(III). Tissue concentrations of As(III), DMA, and MMA in bladder increased dramatically after chronic dosing. However, after washout for 30 days, As(III), DMA, and MMA were all undetectable in bladder and liver. However, As(III) in hair and low levels of DMA in lung, kidney, heart and hair were still detected. In conclusion, in rabbits we found a similar pharmacological profile after a single dose or chronic multiple dosing of parenteral arsenic trioxide, with a limiting metabolizing capacity at a dose of 1.5 mg/kg. Tissue accumulation of arsenic species, mainly DMA, and its reversibility after washout were tissue-selective. The potential for late toxicities of arsenic trioxide in organs with a significant tendency for arsenic accumulation with low reversibility should be closely monitored.

Keywords Arsenic trioxide · Monomethylarsonic acid · Dimethylarsinic acid · Pharmacokinetics

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Introduction

In contrast to their notorious carcinogenic properties in environment pollutants, arsenic compounds have been used for medical purposes for a long time [1]. Recent studies have shown the high effectiveness of parenteral administration (10 mg/day) of arsenic trioxide (As_2O_3) for the treatment of acute promyelocytic leukemia (APL) [2]. The antineoplastic effects of As_2O_3 are related to partial cytodifferentiation and activation of cysteine proteases instrumental in apoptosis. Studies using APL cells and the NB4 cell line have indicated that these antineoplastic effects of As_2O_3 are dose-dependent [3, 4]. As_2O_3 triggered apoptosis at concentrations in the range 0.5–2.0 μM (i.e., 98.9–395.7 ng/ml) and induced partial differentiation at concentrations in the range 0.1–0.5 μM (i.e., 19.8–98.9 ng/ml). However, major adverse effects of As_2O_3, including lethal cardiac dysfunction and liver injury, have been reported [5–9].

The toxicity of arsenic compounds depends on the fractions of different chemical species of arsenics and on the various degrees of methylation. In humans, inorganic arsenic is methylated to monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and, to lesser extent, trimethylarsine oxide (TMAO). Methylated arsenic species are more rapidly excreted in urine than inorganic arsenics and are generally believed to be less toxic [10, 11]. However, recent evidence indicates that the trivalent intermediates of MMA and DMA may play a role in arsenic toxicity or carcinogenicity [12–17]. Therefore, determination of plasma and tissue concentrations of arsenic compounds after As_2O_3 therapy is mandatory in order to define the therapeutic strategy using As_2O_3.

While the body burden of trivalent inorganic arsenite [As(III)] as a result of environmental pollution or intoxication has been reported, pharmacokinetic data for inorganic and organic arsenics at therapeutic levels after parenteral administration of As_2O_3 are still limited. Shen et al. [2] have reported data for some pharmacokinetic parameters and the arsenic levels in the nail and hair after intravenous infusion of 0.16 mg/kg As_2O_3 in patients with relapse of APL. However, the dose–concentration relationship of As_2O_3 and its metabolites in blood and tissues are still unknown. The New Zealand White rabbit has been shown to have an arsenic methylation process similar to that in humans [18–21]. This rabbit was used in this study as a model to determine blood and tissue concentrations of arsenite and the metabolites (DMA and MMA) after parenteral administration of As_2O_3 starting from therapeutic doses in the range 0.2–1.5 mg/kg. The effects of single and multiple dosing of As_2O_3 were also compared.

Methods

The research committee of our institution approved the experimental design. As_2O_3 injection (Asadin; 1 mg/ml) was kindly provided by TTY Biopharm Company of Taiwan. Standards of As(III) (arsenite) and MMA(V) with purities of at least 99% were purchased from ChemService (West Chester, Pa.). DMA (cacodylic acid) was purchased from Sigma Chemical Company (St Louis, Mo.).

Animal experiments

New Zealand White rabbits weighing 2.1–2.4 kg were used. In the single-dose study, rabbits were given a single 0.2, 0.6, or 1.5 mg/kg dose of As_2O_3 intravenously. Blood samples were collected before dosing and at 15 and 30 s, and at 1, 10, 30, 60, 120, 180, 240, 360, 480 and 720 min after the single dose of As_2O_3. Rabbits were killed after blood sampling was complete and tissue samples were collected.

In the dose-accumulation study, 0.2, 0.6, or 1.5 mg/kg As_2O_3 was administered intravenously to rabbits once daily for 30 days. Blood samples were collected on day 1 and day 30 in the same manner as in the single-dose study. After blood sampling was complete, animals were killed and tissue samples were collected.

To evaluate the elimination of accumulated arsenics in organs after chronic administration of As_2O_3, 0.2 or 0.6 mg/kg of As_2O_3 was given to rabbits intravenously for 30 days followed by another 30 days without any treatment. On day 61 after the start of dosing (30 days after the discontinuation of the chronic administration), animals were killed and tissue samples were collected.

Analysis of arsenic species

The extraction and purification of arsenics were performed according the methods of Gomez-Ariza et al. [22] with little modification. For tissue sample analyses, 0.5 g freeze-dried organs were extracted with 160 ml methanol/water solutions (1/1) using a Soxhlet extraction apparatus for 16 h. After removing the methanol solution, the extracts were freeze-dried to powder and dissolved in 10 ml deionized water. The reconstituted liquids were passed through 6-ml C18 extraction columns (Bakerbond, J.T. Baker) as a purification procedure. Arsenic species including As(III), MMA(V), and DMA(V) were determined according to the methods described by Hsueh et al. [23]. For blood sample analyses, 0.5 ml blood samples were mixed with 4.5 ml deionized water. After 30 min sonication, samples were mixed with 5 ml methanol followed by another 30 min sonication. After centrifugation at 1790 *g* for 10 min, supernatants were evaporated and redissolved in 10 ml deionized water. The redissolved liquids were passed through 6-ml C18 extraction columns. Aliquots of 200 µl organ or blood extracts were injected into a HPLC system (Hitachi 7110, Naka, Japan) equipped with an anion column (Phenomenex, Nucleosil, 10 µm, SB 100A, 250×4.6 mm), and linked to a hydride

generation-atomic absorption spectrophotometer (HG-AAS; FIAS 400/Analyst 100, PerkinElmer) to separate As(III), DMA, and MMA. The mobile phase contained 25 m*M* phosphate buffer solution (pH 5.5) and was pumped at a flow rate of 1.5 ml/min. The elution order was As(III), DMA, and MMA.

Quality assurance and quality control in the laboratory

Samples were spiked with arsenic species to calculate the recovery rate in each extraction step and laboratory procedure. The recovery rates of laboratory procedures for As(III), DMA, and MMA ranged from 93.8% to 102.2% with the detection limits of 0.4, 0.3, and 0.42 ng/ml, respectively. The extraction recovery rate for As(III), MMA, and DMA ranged from 90% to 110%. The intraday and interday values of the coefficient of variation (CV) were less than 5%.

Data analysis

Blood concentration-time curves were analyzed by the noncompartmental method. The terminal plasma concentrations were used to estimate the first-order elimination rate constant (λ_z). The terminal half-life ($T_{1/2\beta}$) was calculated by the ratio of 0.693 over the terminal slope, β. The area under the drug concentration-time curve (AUC) (ng ml^{-1} h) was calculated using the linear trapezoidal rule and by extrapolating time to infinity by dividing the last measurable concentration by λ_z values. The mean residence time (MRT) was calculated as the ratio AUMC over AUC, where AUMC is the area under the first moment (concentration multiplied by time) versus time curve. Total body clearance was expressed as CL and was estimated as D/AUC, where D represents the given dose. Volume of distribution (Vss) was calculated as the product of MRT and CL. The significance of differences were evaluated by ANOVA with a level of significance set at 0.05. Pair-wise comparisons among treatment groups were made using Tukey's test.

Results

Arsenic species in the blood

After single dose of As_2O_3 of 0.2, 0.6 or 1.5 mg/kg was administered to rabbits, only As(III) and trace amounts of DMA were detected in the blood (Fig. 1). On day 1, total body clearance significantly decreased as the dose increased (Table 1). Accordingly, the AUC increased significantly as the dose increased. When AUC values were normalized by the given doses, it was noted that the increase in AUC was disproportional to the dose. The AUC/D ratio slightly increased from the dose of 0.2 mg/kg to the dose of 0.6 mg/kg, while the ratio at a dose of 1.5 mg/kg was nearly double that at 0.2 mg/kg (1.88 ± 0.13 vs 1.07 ± 0.18). The decrease in clearance and the subsequent increase in AUC indicate that the capacity for the elimination of As(III) would reach a limit with the increasing doses, and that this limit had already been reached at a dose of 1.5 mg/kg. Subsequently, the MRT of As(III) increased as the dose increased, reaching the highest value at the dose of 1.5 mg/kg. Although not statistically significant, the half-life of As(III) tended to increase as the dose increased. On the other hand, the volume of distribution (Vss) appeared to be unaffected by the dose. Since Vss reflects the relationship between dose and blood levels, the lack of change in Vss suggests that the tissues provide a reservoir for the distribution of As(III)

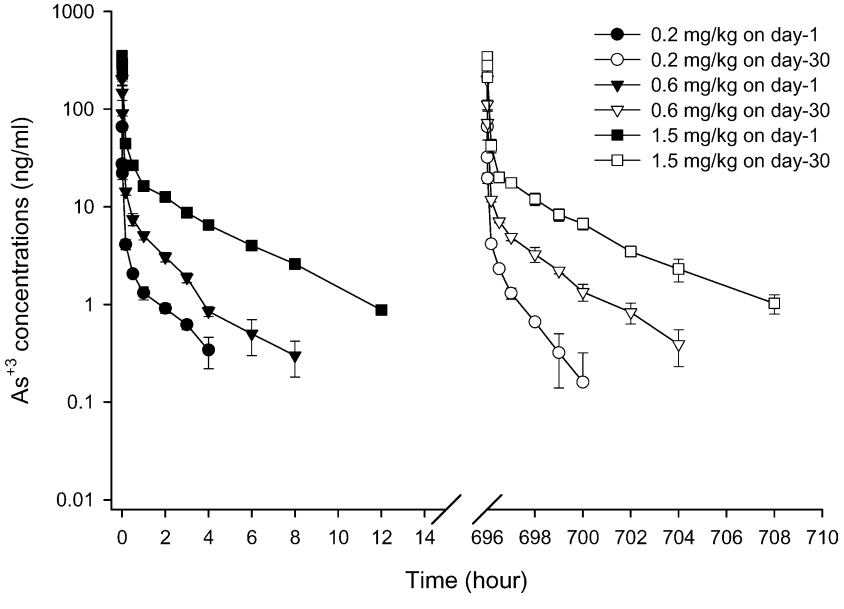

Fig. 1 Blood concentrations of As(III) in rabbits given As_2O_3 at 0.2, 0.6, and 1.5 mg/kg on day 1 and daily for 30 days. The data are presented as means ± SE ($n = 4$)

Table 1 Pharmacokinetic properties of As(III) after a single and multiple intravenous administrations of As_2O_3. The values presented are means ± SD from four rabbits

	Dose		
	Group 1 (0.2 mg/kg)	Group 2 (0.6 mg/kg)	Group 3 (1.5 mg/kg)
Day 1			
AUC/D (min/l)	1.07 ± 0.18	1.26 ± 0.19	1.88 ± 0.13[a]
CL (ml/min)	956 ± 145	810 ± 114	538 ± 44[b]
Vss (l)	99 ± 21	77 ± 23	112 ± 24
MRT (min)	105 ± 22	98 ± 41	209 ± 42[c]
$T_{1/2\beta}$ (min)	118 ± 17	139 ± 87	163 ± 12
Day 30			
AUC/D (min/l)	1.02 ± 0.11	1.23 ± 0.34	1.75 ± 0.34[d]
CL (ml/min)	989 ± 114	860 ± 265	587 ± 111[e]
Vss (l)	120 ± 68	97 ± 21	115 ± 22
MRT (min)	127 ± 83	117 ± 29	199 ± 34
$T_{1/2\beta}$ (min)	149 ± 93	125 ± 41	175 ± 26

[a] $P < 0.001$ compared to group 1 and $P < 0.01$ compared to group 2 day 1
[b] $P < 0.01$ compared to group 1 and $P < 0.05$ compared to group 2 day 1
[c] $P < 0.01$ compared to group 1 or group 2 day 1
[d] $P < 0.05$ compared to group 1 day 30
[e] $P < 0.05$ compared to group 1 day 30

to compensate for changes in the dose/concentration ratio. This phenomenon was corroborated by the data collected on day 30.

After daily dosing for 30 days, blood levels and pharmacokinetic parameters of As(III) remained unchanged compared to those on day 1 (Fig. 1) which suggests that either the accumulation of As(III) in tissues was negligible or tissues provided an adequate buffering effect for As(III) distribution. The latter possibility was supported by the following tissue data.

Arsenic species in the organs

In contrast to blood data in which As(III) was the only detectable chemical species, As(III), MMA, and DMA were all detected in organ tissues and DMA levels were severalfold higher than the As(III) and MMA levels. In samples from both day 1 and day 30, tissue distributions of the three arsenic species in general increased with dose, with the increase most significant at a dose of 1.5 mg/kg. When tissue contents of arsenic species were normalized by the doses (Fig. 2), the dose-distribution relationships for most arsenic species appeared to be linear, suggesting that sufficient tissue capacity could be provided under the current dose range. However, nonlinear dose effects for the tissue distribution were observed for As(III) in kidney on day 1 and for DMA in liver on day 30 after multiple dosing.

As shown in Table 2, on day 1 after the single dose, spleen contained the highest concentration of As(III) followed by hair, liver, lung, kidney, heart, bladder, and bone. However, at the highest test dose (1.5 mg/kg), the tissue concentration of As(III) was highest in the kidney and that in bladder was only lower than in kidney, hair, and liver. The dose-distribution relationships obtained after normalizing the tissue contents by the doses were nonlinear in the kidney on the day 1 after a single dose of As_2O_3 (Fig. 2). After multiple dosing for 30 days, the accumulation of As(III) was evident in bladder (more than threefold). The tissue accumulation of As(III) in hair and heart also showed a trend of tissue accumulation after chronic multiple dosing, but to a lower extent than in bladder. On the contrary, As(III) concentrations in kidney and to a lower extent in liver were even lower after chronic multiple dosing than after a first single dose.

The distribution of MMA is summarized in Table 3. On day 1, after a single dose, MMA could only be detected in lung after dosing at 0.2 mg/kg, but MMA could be detected in the lung as well as kidney, liver, heart, and bladder at higher doses of As_2O_3 (0.6 and 1.5 mg/kg). On day 30 after multiple dosing, organs including liver, kidney, bladder, and heart that initially did not have detectable MMA on day 1 after a single dose (0.2 and 0.6 mg/kg) were found to have detectable levels of MMA. Significant accumulation of MMA caused by multiple dosing was observed in bladder and liver. The tissues with high tissue contents of As(III), including spleen and hair, showed no or very low levels of MMA and DMA.

On day 1 after single dose of As_2O_3, the tissue concentrations of DMA were much higher than those of As(III) and MMA, being highest in lung, followed by liver, kidney, heart and spleen (Table 4). In general, the tissue concentrations of DMA of most organs were higher after multiple dosing than after a single dose. However, similar to As(III) and MMA, it was noted that the accumulation of DMA in bladder after multiple dosing was more prominent than in other organs. In hair and bone, only after chronic multiple dosing could DMA be found. The dose-distribution relationship of DMA was found to be nonlinear in liver on day 30 after chronic multiple dosing.

Tissue distribution of arsenic compounds after washout

Arsenic species, including As(III), MMA, and DMA, had decreased to undetectable levels in most organs by day 60 after the 30-day washout period following the chronic administration of As_2O_3, but appreciable concentrations of As(III) were still found in hair (Table 2). As for the major tissue metabolite, DMA, it could still be detected in lung, hair, kidney and heart (Table 4). MMA was detected in kidney. Surprisingly, in bladder, that was associated with the most significant tissue accumulation of As(III), MMA, and DMA after chronic multiple dosing, As(III), DMA, and MMA were all cleared to undetected levels. A similar phenomenon was also observed in liver (only low levels of As(III) detected).

Discussion

Parenteral As_2O_3 therapy is highly effective for the induction of remission in adults or children with promyelocytic leukemia. However, the pharmacokinetic characteristics of such therapy, especially after multiple dosing, are far from clear. The main results of this study using a rabbit model were as follows: (1) a decreased clearance of As(III) and nonlinear blood levels of As(III) after a higher single dose (1.5 mg/kg) of As_2O_3 suggest the existence of saturable enzyme systems which catalyze As(III); (2) after chronic dosing of As_2O_3, the pharmacokinetic parameters and blood levels of As(III) remained unchanged; (3) DMA, rather than As(III) and MMA, was the major arsenic compound in tissues; and (4) DMA, MMA as well as As(III) accumulated with tissue selectivity after multiple chronic parenteral As_2O_3 administration and could be washout completely (e.g., in bladder), partially (e.g., in liver, heart, lung, and kidney) or minimally (e.g., in hair). Although great care must be taken in the direct extrapolation of results from experimental studies involving animal models to clinical therapy, our data are important references for therapy using As_2O_3.

Total body clearance is a measure of elimination from the body. Elimination can be attributed to metabolism and excretion. In terms of metabolism, in most mammals, inorganic arsenic introduced into the body is methylated to MMA and then to DMA. Methylation of inorganic arsenics occurs via alternating reduction of pentavalent species to trivalent species followed by the addition of a methyl group. The initial sequence in the

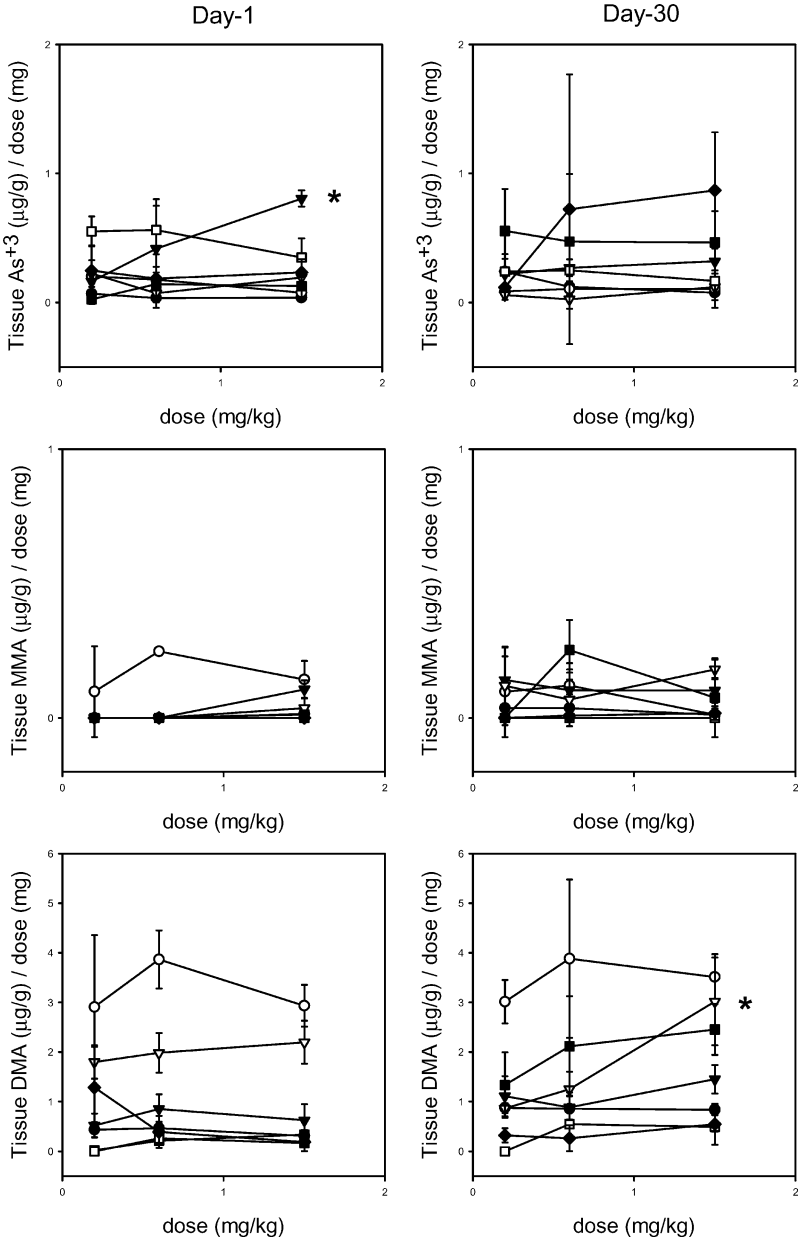

Fig. 2 Normalized tissue distributions of As(III), MMA, and DMA by doses in heart (*filled circles*), lung (*open circles*), kidney (*filled down triangles*), liver (*open down triangles*), bladder (*filled squares*), spleen (*open squares*), and hair (*filled diamonds*) after 1 day and 30 days of dosing. The data are presented as means ± SD. $^*P < 0.05$

Table 2 Tissue distributions of As(III) after a single (1 day) and multiple intravenous (30 days) administrations of As_2O_3. A 30-day recovery period was provided for rabbits receiving 30-day multiple dosing (day 60). The values presented are means ± SD micrograms per gram dry tissue from three rabbits (*UD* undetectable)

	Dose		
	Group 1 (0.2 mg/kg)	Group 2 (0.6 mg/kg)	Group 3 (1.5 mg/kg)
Day 1			
Heart	0.013 ± 0.016	0.021 ± 0.021	0.054 ± 0.038
Lung	0.040 ± 0.005	0.106 ± 0.059	0.113 ± 0.052
Kidney	0.032 ± 0.020	0.250 ± 0.230	1.208 ± 0.095
Liver	0.045 ± 0.020	0.043 ± 0.014	0.289 ± 0.052
Bladder	0.005 ± 0.003	0.084 ± 0.055	0.193 ± 0.149
Spleen	0.110 ± 0.023	0.336 ± 0.112	0.522 ± 0.222
Bone	UD	UD	0.039 ± 0.019
Hair	0.049 ± 0.039	0.110 ± 0.136	0.348 ± 0.156
Day 30			
Heart	0.048 ± 0.005	0.072 ± 0.050	0.114 ± 0.021
Lung	0.017 ± 0.004	0.062 ± 0.036	0.155 ± 0.219
Kidney	0.044 ± 0.031	0.161 ± 0.126	0.476 ± 0.181
Liver	0.011 ± 0.007	0.014 ± 0.003	0.178 ± 0.086
Bladder	0.111 ± 0.065	0.284 ± 0.313	0.698 ± 0.363
Spleen	0.048 ± 0.020	0.150 ± 0.051	0.251 ± 0.223
Bone	UD	UD	0.025 ± 0.016
Hair	0.023 ± 0.019	0.433 ± 0.628	1.303 ± 0.675
Day 60			
Heart	UD	UD	
Lung	UD	UD	
Kidney	0.005 ± 0.003	0.011 ± 0.013	
Liver	0.001 ± 0.002	0.015 ± 0.023	
Bladder	UD	0.001 ± 0.002	
Spleen	UD	UD	
Bone	UD	UD	
Hair	0.098 ± 0.090	0.186 ± 0.134	

Table 3 Tissue distributions of MMA(V) after a single (1 day) and multiple intravenous (30 days) administrations of As_2O_3. A 30-day recovery period was provided for rabbits receiving 30-day multiple dosing (day 60). The presented are means ± SD micrograms per gram dry tissue from three rabbits (*UD* undetectable)

	Dose		
	Group 1 (0.2 mg/kg)	Group 2 (0.6 mg/kg)	Group 3 (1.5 mg/kg)
Day 1			
Heart	UD	0.014 ± 0.024	0.023 ± 0.023
Lung	0.020 ± 0.033	0.149 ± 0.008	0.215 ± 0.103
Kidney	UD	UD	0.159 ± 0.050
Liver	UD	UD	0.055 ± 0.053
Bladder	UD	UD	0.018 ± 0.016
Spleen	UD	UD	UD
Bone	UD	UD	UD
Hair	UD	UD	UD
Day 30			
Heart	0.007 ± 0.012	0.022 ± 0.030	0.018 ± 0.035
Lung	0.019 ± 0.033	0.073 ± 0.050	0.311 ± 0.139
Kidney	0.028 ± 0.024	0.062 ± 0.047	0.154 ± 0.069
Liver	0.024 ± 0.022	0.041 ± 0.060	0.271 ± 0.055
Bladder	UD	0.152 ± 0.066	0.113 ± 0.220
Spleen	UD	UD	UD
Bone	UD	UD	UD
Hair	UD	0.005 ± 0.010	0.008 ± 0.016
Day 60			
Heart	UD	UD	
Lung	UD	UD	
Kidney	0.009 ± 0.011	0.022 ± 0.026	
Liver	UD	UD	
Bladder	UD	UD	
Spleen	UD	0.060 ± 0.002	
Bone	UD	UD	
Hair	UD	UD	

biotransformation process of different arsenic species would be As(III) followed by MMA(V), MMA(III), DMA(V), and DMA(III) or further. Diversity in the metabolizing capacity for the conversion of As(III) and its metabolites between species and organs has been reported. Although the enzymes involved in the metabolism of arsenics are still unclear, it has been suggested that glutathione (GSH) and probably other thiols serve as reducing agents for pentavalent species. S-Adenosylmethionine (SAM) mediates the transfer of the methyl group [24]. The cellular toxicity caused by arsenic species is inversely related to intracellular GSH levels and can be enhanced by GSH depletion [25, 26]. These enzyme activities have been detected in liver, kidney and lung [24]. In addition to metabolism, it has been shown that the efflux of arsenics can be mediated by a variety of membrane transporters, including P-glycoprotein and multidrug resistance-associated protein [27, 28]. Although further elucidation would be required, the saturation of these carrier-mediated processes might result in reduction of clearance and accumulation of arsenics in the body. We observed that, after a single dose of As_2O_3, the blood levels of As(III) increased nonlinearly and the clearance of As(III) decreased at the highest tested dose of As_2O_3. These parameters, however, were essentially the same after chronic multiple dosing. These findings suggest that the capacity for the elimination of As(III) from the blood reaches a limit with increasing doses to 1.5 mg/kg. After chronic multiple dosing of As_2O_3, this limit remains the same but tissues may still provide adequate buffering effects for chronically administered As(III) and lead to a similar pharmacokinetic profile of As(III). Since DMA was found to be the major arsenic compound in tissues after single or chronic multiple dosing of As_2O_3, the efficiency of methylating As(III) to DMA should be high enough to account for this buffering effect.

In kidney, we observed a nonlinear dose-distribution relationship for tissue As(III) after a single first dose of As_2O_3, that disappeared after chronic multiple dosing. Furthermore, the tissue concentration of As(III) after chronic multiple dosing was even lower than that after a single first dose. Along with higher levels of DMA in kidney after chronic multiple dosing than a single dose, we may suggest that the capacity of methylating As(III) to DMA is inducible in kidney after chronic multiple dosing. However, the washout of As(III), DMA, and MMA in kidney after discontinuation of As_2O_3 was incomplete and possibly slow and therefore these species could be detected in kidney tissues. Hence, the admin-

Table 4 Tissue distributions of DMA(V) after a single (1 day) and multiple intravenous (30 days) administrations of AS_2O_3. A 30-day recovery period was provided for rabbits receiving 30-day multiple dosing (day 60). The values presented are means ± SD micrograms per gram dry tissue from three rabbits (*UD* undetectable)

	Dose		
	Group 1 (0.2 mg/kg)	Group 2 (0.6 mg/kg)	Group 3 (1.5 mg/kg)
Day 1			
Heart	0.087 ± 0.030	0.278 ± 0.078	0.463 ± 0.019
Lung	0.581 ± 0.289	2.318 ± 0.352	4.399 ± 0.633
Kidney	0.104 ± 0.148	0.511 ± 0.178	0.938 ± 0.482
Liver	0.360 ± 0.067	1.190 ± 0.240	3.295 ± 0.649
Bladder	0.004 ± 0.005	0.128 ± 0.004	0.508 ± 0.131
Spleen	UD	0.155 ± 0.004	0.248 ± 0.244
Bone	UD	UD	UD
Hair	UD	UD	0.172 ± 0.024
Day 30			
Heart	0.175 ± 0.019	0.513 ± 0.197	1.253 ± 0.166
Lung	0.603 ± 0.087	2.328 ± 0.958	5.269 ± 0.694
Kidney	0.222 ± 0.081	0.530 ± 0.143	2.177 ± 0.429
Liver	0.172 ± 0.018	0.750 ± 0.211	4.528 ± 1.326
Bladder	0.267 ± 0.132	1.270 ± 0.604	3.683 ± 0.770
Spleen	UD	0.328 ± 0.185	0.739 ± 0.151
Bone	0.025 ± 0.039	0.069 ± 0.060	0.137 ± 0.032
Hair	0.064 ± 0.029	0.157 ± 0.156	0.817 ± 0.621
Day 60			
Heart	0.003 ± 0.007	0.008 ± 0.003	
Lung	0.075 ± 0.015	0.100 ± 0.027	
Kidney	0.019 ± 0.015	0.065 ± 0.023	
Liver	UD	UD	
Bladder	UD	UD	
Spleen	UD	0.065 ± 0.002	
Bone	UD	UD	
Hair	0.019 ± 0.014	0.066 ± 0.018	

istration of As_2O_3 at high doses for sustained periods may better be avoided in patients with compromised renal function.

Our results also showed that the tissues, including spleen and hair, initially contained high concentrations of As(III) but had very low levels of DMA and MMA. Significant tissue accumulation of As(III) after chronic multiple dosing in hair was also noted. It may be deduced that the metabolizing system for As(III) in hair and spleen is of low activity. The clearance of As(III), DMA, and MMA after cessation of chronic As_2O_3 administration was also very slow in hair. This observation echoed the that of a previous studies of chronic tissue accumulation of arsenic compounds in hair after the ingestion of arsenics [29]. However, in spleen possibly due to an adequate splenic blood flow, the clearance was complete after washout for 30 days. On the other hand, liver and lung, that are known to be important sites of arsenic methylation, contained the highest levels of DMA and MMA. A previous study in liver epithelial cells has also shown that after continuous exposure to 500 n *M* As(III) for 18–20 weeks, the DMA/As ratio is significantly increased and suggested an inducible metabolic pathway for the conversion of As(III) to DMA in liver [30]. Our findings for liver tissue similarly showed decreased As(III) concentration and an increased DMA/As ratio after chronic multiple dosing of As_2O_3. However, the dose-distribution relation for DMA after chronic multiple dosing in liver was still nonlinear and suggested a limit in eliminating DMA after a chronic high load of As(III). Whether this limit is related to the observed hepatotoxicities after chronic As_2O_3 therapy remains to be elucidated [2, 7]. Nonetheless, our results indicate that the accumulated As(III), MMA, and DMA in liver after chronic multiple dosing could largely be cleared after cessation of chronic As_2O_3 administration.

Chronic As_2O_3 therapy may also be associated with cardiac toxicity. Our previous results in rabbits showed no discernible immediate cardiac effects after As_2O_3. However, chronic As_2O_3 administration resulted in a prolonged ventricular repolarization and the development of ventricular tachyarrhythmias [29]. These chronic cardiac electrophysiological effects were partially reversible after cessation of the chronic As_2O_3 administration. In this study, we found that tissue accumulation of As(III) and DMA after chronic As_2O_3 administration was evident in heart, but after washout for 30 days, only DMA was detected in the heart. These findings may imply that not only As(III) but also DMA may affect the cardiac electrophysiological properties and play different roles in cardiac toxicity at different stages of As_2O_3 therapy.

We also identified a strong reversibility of tissue accumulation for As(III), MMA, and DMA in bladder. The bladder showed the most significant accumulation of As(III), MMA, and DMA after chronic As_2O_3 administration of the organs examined, but all the accumulated arsenic compounds were cleared after washout for 30 days. Previous studies have emphasized an association between the risk of bladder cancer and arsenic in drinking water [31]. A significant dose-response relationship between risk of transitional call carcinoma and indices of arsenic exposure has been observed even after adjustment for age, sex, and cigarette smoking [32]. However, our results showed that the accumulation of arsenic compounds in the bladder after chronic multiple dosing would be cleared after cessation of dosing. A similar reversibility of the accumulation of arsenic compounds in the liver was also demonstrated. In addition, a partial reversibility (low but still detectable levels of arsenic compounds after washout) of accumulation in lung, kidney and heart was found. The actions and potential toxicities of arsenic are dependent on the arsenic species, the length and dose of exposure and the cell type [33]. Based on our data, a washout phase should be important in reducing the potential toxicities of chronic As_2O_3 therapy for APL or other solid cancers.

The carcinogenic effects of arsenic could be due to the activation of transcription factors such as the AP-1 family [34, 35]. However, recent studies have shown that trivalent methylated arsenicals such as MMA(III) and DMA(III) may be even more toxic than As(III) [36–38]. The DMA-associated organ-specific toxic effects have

been attributed to the formation of peroxyl radicals along with other active oxygen species [12], as well as the induction of single-strand DNA breaks and DNA-protein crosslinks [39]. DMA-induced toxicity or carcinogenicity has been observed in lung, bladder, kidney, and liver in rodents [13, 15, 17]. Our results showed that, in contrast to blood where As(III) was the major measurable arsenic compound, DMA was the major metabolite in organs. Since it is the pentavalent DMA that was determined in the current study, the results imply that the preceding arsenic species such as trivalent MMA may have been equally present in these tissues. Nonetheless, the roles played by the methylated metabolites of arsenic in the toxicities of As_2O_3 treatment need to be defined by further studies.

The mechanisms responsible for the effectiveness of As_2O_3 therapy in the treatment of the malignancies are still not very clear. In primary cell cultures of APL, As_2O_3 triggers apoptosis at concentrations in the range 98.9–395.7 ng/ml and induces partial differentiation at concentrations in the range 19.8–98.9 ng/ml [3, 4]. Since As_2O_3 consists of about 76% As(III) by weight, assuming As(III) is mainly responsible for the effects, the corresponding As(III) concentrations would be about 75–300 ng/ml for apoptosis and about 15–75 ng/ml for partial differentiation. Based on our results regarding the blood levels of arsenic species, after a dose of 1.5 mg/kg, the blood levels of As(III) would stay within the therapeutic range for a longer period. The clinical significance of this observation may be elucidated by further studies.

In conclusion, this study in rabbits demonstrated nonlinear blood levels of As(III) following parenteral administration of As_2O_3 in the dose range 0.2–1.5 mg/kg and indicated saturable although efficient metabolizing enzyme systems to convert As(III) to DMA. Thus, DMA was the major metabolite in tissue after As_2O_3 therapy. Nonetheless, the over-saturation sustained at high doses of As_2O_3 may be compensated by enzyme induction in certain tissues (e.g., kidney). The tissue accumulation of arsenic compounds and its reversibility after washout were tissue-selective. The potential for late toxicities of As_2O_3 in organs with a significant tendency for arsenic accumulation and low reversibility should be closely monitored.

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