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ORIGINAL ARTICLE

Pharmacokinetics of arsenic species in Japanese patients with relapsed or refractory acute promyelocytic leukemia treated with arsenic trioxide

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Abstract
Purpose To investigate the pharmacokinetics of arsenic species in Japanese patients with relapsed or refractory acute promyelocytic leukemia (APL) treated with arsenic trioxide (ATO) at a daily dose of 0.15 mg/kg.
Methods Inorganic arsenic (As^{III} and As^{V}) and the major metabolites monomethylarsonic acid (MAA^{V}) and dimethylarsinic acid ($DMAA^{V}$) in plasma and urine collected from 12 Japanese patients were quantified by HPLC/ICP-MS.
Results The plasma concentrations of As^{III} and As^{V} on day 1 reached the similar *C*max (12.4 ± 8.4 and 10.2 ± 3.9 ng/ml) immediately after completion of administration followed by a biphasic elimination. The $AUC_{0-\infty}$ of As^{V} was about twice that of As^{III}. The appearance of methylated metabolites in the blood was delayed. During the repeated administration, the plasma concentrations of inorganic arsenic reached the steady state. In contrast, the MAA^{V} and $DMAA^{V}$ concentrations increased in relation to increased administration frequency. The mean total arsenic excretion rate including inorganic arsenic and methylated arsenic was about 20% of daily dose on day1 and remained at about 60% of daily dose during week 1–4.
Conclusions This study demonstrates that ATO is metabolized when administered intravenously to APL patients and methylated metabolites are promptly eliminated from the blood and excreted into urine after completion of administration, indicating no measurable accumulation of ATO in the blood.

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Introduction

Acute promyelocytic leukemia (APL) is a distinctive type of acute myelocytic leukemia (AML) characterized by chromosome translocations *t* (15; 17) and accounts for approximately 10–15% of all cases of AML. In the 1990s, investigators from China reported that arsenic trioxide (ATO) induces complete remission (CR) in patients with relapsed or refractory APL

[1–3]. After the Chinese reports, the pilot and multicenter studies of ATO were conducted in the United States in relapsed or refractory APL patients. Combining the results from the pilot and multicenter studies, the CR rate was 87% [4, 5]. At present, ATO is the first-choice treatment of relapsed or refractory APL. In Japan, we have previously reported the prospective study of ATO in APL patients [6]. ATO was administered by the same protocol used in the United States multicenter study [5] to 14 relapsed APL patients who had not responded to ATRA and conventional chemotherapy, and of these 11 (78%) achieved CR.

While the clinical effect of ATO against APL was established, its pharmacokinetics has yet to be clarified. ATO is methylated by methyl transferase in the liver to monomethyl and dimethyl arsenic compounds, including the major metabolites monomethylarsonic acid (MAA^V) and dimethylarsinic acid ($DMAA^V$), which are mostly excreted into urine [7, 8]. Recent investigation shows that, among inorganic arsenic and methylated metabolites, As^{III} could induce cytotoxity against NB4 cells that derived from APL, degradation of PML-RARα chimeric protein that formed as a consequence of the t (15; 17) and causes the pathogenesis of APL, and differentiation in NB4 cells [9]. However, in most reports on the pharmacokinetics of ATO to patients with APL and other hematological malignancies, the arsenic concentrations were measured as total arsenic including metabolites [3, 10–14]. Recently, since the HPLC/ICP-MS, which combines high performance liquid chromatography (HPLC) and inductivity coupled plasma mass spectrometry (ICP-MS), was used for the quantitation of arsenic, the pharmacokinetics of arsenic species has been analyzed in several reports [15, 16]. However, the pharmacokinetic data of arsenic species for Asian people are presently limited [16].

In the prospective study [6], we have collected the blood and urine from 12 APL patients, subsequently stored frozen. Using the freeze-stored samples, we determined inorganic arsenic (As^{III} and As^V) and the major metabolites MAA^V and $DMAA^V$ concentrations by the HPLC/ICP-MS method [16], and conducted a pharmacokinetic analysis.

Patients and methods

Patients and administration schedule

The prospective study [6] was conducted at the Hamamatsu University School of Medicine in 14 APL patients from March 1999 to March 2001 according to the United States multicenter study [5]. ATO (Trisenox®) was provided by PolaRx Biopharmaceuticals (New York, NY, USA) and later from Cell Therapeutics (Seattle, WA, USA). Eligibility criteria and treatment plan were previously described. [6] The protocol was reviewed and approved by the institutional review board of the Hamamatsu University Hospital. Written informed consent was obtained from patients before the treatment. During the induction treatment, ATO was administered intravenously over 2 h at a dose of 0.15 mg/kg in 500 ml of 5% of dextrose given once daily for cumulative maximum of 60 days. Patients who had achieved complete remission received one course of consolidation therapy with ATO for a cumulative total of 25 days, using the same dose and schedule as the induction therapy.

Samples used in the pharmacokinetic analysis

The blood and urine were collected from 12 patients during the induction treatment. Samples were also collected from two patients (patient 7 and 9) during the consolidation treatment. Sample of blood of 5 ml was collected on the first day of administration (day 1) and after 1, 2, and 4 weeks from the start of administration. The time points for blood collection were as follows. On day 1 and after 4 weeks: before administration, 1 h, at the end of the infusion, 4, 6, 12 (or 18) and 24 h after the start of administration. After 1 and 2 weeks: before administration, at the end of the infusion, and 4 h after the start of administration.

The urine was collected for 24 h on day 1, and after 1, 2, and 4 weeks. After each urine sample collection, urine volume was measured. The plasma obtained from blood and urine samples were stored frozen (–20 or –80°C) until analysis.

As the standard arsenic compounds, sodium arsenite (As^{III}), sodium arsenate (As^V), monomethylarsonic acid (MAA^V), dimethylarsinic acid ($DMAA^V$), trimethyl arsenoxide (TMAsO), arsenobetaine (AB), arsenocholine (AsCho), tetramethylarsonium (TetMAs) and arseno-sugar (AsS) were purchased from Tri Chemical Laboratories Inc. (Yamanashi, Japan).

HPLC/ICP–MS analysis

The quantification of arsenic was performed by HPLC/ICP–MS, which combines HPLC (LC PU611 VS GL Sciences Inc., Tokyo, Japan) and ICP-MS (ELAN DRC-*e* Perkin Elmer SCIEX Inc., Ontario, Canada). [16] Inertsil AS (150 mm × 2.1 mm, 3.0 mm; GL Sciences Inc.) was used as the HPLC column and an

ODS guard column (GL Sciences Inc.) was attached to allow direct injection of the biological samples. Plasma samples were prepared using a protein precipitation procedure with acetonitrile. Urine samples were diluted twice with column eluate (dilution factor = 1:1). Column eluate was developed with 10 mM sodium butanesulfonate, 4 mM tetramethyl ammonium hydroxide, 4 mM malonic acid, and 0.05% methanol at pH 3.0 with HNO_3 and the elution flow rate used was 0.2 ml/min. All samples were filtered using a 0.45 μm membrane filter before injection.

The arsenic detection was performed at m/z 75 by ICP-MS. Sample solution of 5 μl was injected onto the guard column and the amount of each arsenic compound was obtained from the calibration curve (the standard arsenic compound was diluted with water to 1, 5, 10, and 20 ppb As). The lower limit of quantification for each arsenic species (As^{III}, As^{V} and the methylated metabolites) was 0.1 ppb.

Pharmacokinetic analysis

On the basis of plasma concentrations, the following pharmacokinetic parameters were calculated by non-compartmental analysis. It was not possible to calculate the parameters in some patients due to incomplete blood sampling. For inorganic arsenic, the parameters on day 1 were obtained from 10–11 patients and those on week 4 obtained from six patients. For the methylated metabolites, the C_{max} and T_{max} were obtained from 11–12 patients and $AUC_{0-tlast}$ was obtained from 9 patients on day 1 while these parameters were determined in six patients on week 4.

1. Maximum concentration (C_{max}) and time to reach C_{max} (T_{max}): C_{max} and T_{max} were obtained from the measured values.
2. Elimination rate constant (λz): λz was obtained by linear regression of the linear part of the log plasma concentration-time curve in the elimination phase according to the least squares method. The data points (≥ 3 time points) were used to perform linear regression and the slope of the fitted line with the best correlation coefficient was used as the elimination rate constant.
3. Elimination half-life ($t_{1/2,\beta}$): $t_{1/2,\beta}$ was calculated as $Ln(2)/\lambda z$.
4. Area under the plasma concentration-time curve (AUC): the AUC up to the final measurable time point (t_{last}) was calculated by the trapezoidal method ($AUC_{0-tlast}$) and added to $C_{tlast}/\lambda z$ to calculate $AUC_{0-\infty}$, where C_{tlast} is the concentration at t_{last}.
5. Total clearance (CL_{tot}): CL_{tot} was calculated as dose/$AUC_{0-\infty}$ on day 1 or dose/$AUC_{0-tlast}$ on week 4.
6. Volume of distribution (Vz, Vss): Vz was calculated from dose/($\lambda z \times AUC_{0-\infty}$) and Vss from dose × MRT/$AUC_{0-\infty}$. The $AUMC_{0-tlast}$ up to t_{last} ($= \int_0^t t \times Cdt$) was calculated by the trapezoidal method and added to $Ct/kel^2 + Ct \times t/kel$ to calculate $AUMC_{0-\infty}$ and MRT was obtained from $AUMC_{0-\infty}/AUC_{0-\infty}$.

On the basis of urinary concentrations, the urinary excretion (% of dose) and the renal clearance (CL_{re}) were calculated from the following equations:

$$\text{Urinary excretion (\%)} = \frac{\text{Urinary concentration } (\mu\text{g/ml}) \times \text{Total volume (ml)}}{\text{Dose } (\mu\text{g/body})} \times 100$$

$$\text{Renal clearance (l/kg h)} = \frac{\text{Urinary excretion } (\mu\text{g})/\text{body weight (kg)}}{\text{AUC (ng h/ml)}}$$

WinNonlin standard version 4.1 (Pharsight Co., Palo Alto, CA, USA) was used for the non-compartmental analysis. Other calculations were performed using Microsoft Excel 2000 (Microsoft Co., Redmond, WA, USA).

Results

Patient background and efficacy results

The background and outcomes of ATO therapy for 12 APL patients are shown in Table 1. Ten of 12 patients achieved complete remission. Six of 10 patients who achieved CR became negative in the post-treatment RT-PCR test. Patient 11 died of

Table 1 Patient background and efficacy results

Patient number	Age	Sex	As_2O_3 treatment (days)		Outcome	RT-PCR for PML-RARα[b]	CR duration (month)
			Induction	Consolidation			
1	36	F	54	25	CR	–	22+
2	61	M	40	25	CR	–	10
3	36	F	46	20	CR	–	12+
4	33	F	41	25	CR	–	12+
5	58	M	41	25	CR	–	9+
6	52	M	43	–	CR	+	6
7	57	M	43	25	CR	+	12
8	23	F	27	25	CR	–	8
9	62	M	60	25	CR	+	8
10	50	M	44	–	CR	ND	4
11	65	M	21	–	Early death[a]	NA	–
12	64	M	64	–	NR	NA	–

ND not done; *NA* not applicable; *CR* complete remission; *NR* no response

[a] Due to cerebral hemorrhage on day 21

[b] Reverse transcriptase (RT)-PCR assays of bone marrow mononuclear cells for PML-RARα were performed after the consolidation treatment

cerebral hemorrhage on day 21, and patient 12 was discontinued the administration on day 92 because ATO was ineffective.

For two patients (patient 7 and 8) the blood and urine were collected also during the consolidation treatment.

Pharmacokinetics of arsenic species on the first day of administration

The plasma concentrations of inorganic arsenic (As^{III} and As^{V}) on day 1 are shown in Fig. 1, and the pharmacokinetic parameters in Table 2. The plasma concentration of As^{III} reached C_{max} immediately after the administration in most of the patients, followed by a biphasic decline with a mean $t_{1/2,\beta}$ of 17 h after an initial distribution phase (up to 4–8 h after the start of administration). The mean C_{max} was 12.4 ± 8.4 ng/ml. The mean volume of distribution at steady state (Vss) was large (55.9 l/kg) suggesting extensive distribution throughout the body. The plasma concentration profile of As^{V} was similar to that of As^{III}. After reaching C_{max} of 10.2 ± 3.9 ng/ml, a biphasic decline was observed with a mean $t_{1/2,\beta}$ of 18.3 h. After the end of the infusion, the As^{V} plasma concentrations initially declined more slowly than those for As^{III}, and hence the $AUC_{0-\infty}$ of As^{V} was about twice that of As^{III} (As^{III}, 80.5 ± 39.8 ng h/ml; As^{V}, 155.1 ± 78.6 ng h/ml). In addition, the pharmacokinetic parameters for the inorganic arsenic concentrations (the total measured As^{III} and As^{V} values) were calculated, and the results indicated a C_{max} of 22.6 ± 11.4 ng/ml and $AUC_{0-\infty}$ of 211.8 ± 55.1 ng h/ml.

The plasma concentrations and the pharmacokinetic parameters of major metabolites (MAA^{V}, $DMAA^{V}$) on day 1 are shown in Fig. 2 and Table 2, being compared to the inorganic arsenic (As^{III} + As^{V}). The MAA^{V} and $DMAA^{V}$ concentrations were below the quantification limit until immediately after completion of administration, but a gradual increase was observed from 4 h after the start of administration reaching C_{max} in many patients at 24 h after administration, the final time point on day 1. The mean C_{max} values were 3.1 ± 1.6 and 5.4 ± 2.9 ng/ml, respectively. The plasma concentrations of arsenobetaine (AB), an organic

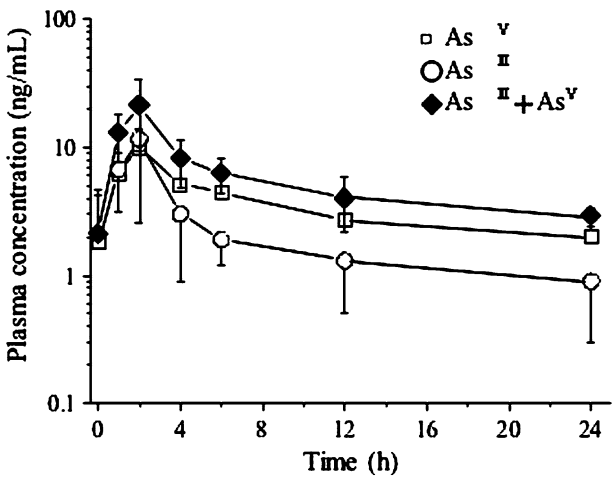

Fig. 1 Plasma concentrations of inorganic arsenic on day 1 of the repeated administration. The values shown in the figure were determined in 12 patients (N = 14; mean ± standard deviation). The values obtained during the induction and consolidation treatment were used for patient 7 and 8

Table 2 Pharmacokinetic parameters of inorganic arsenic (As^{III} + As^{V}) and its metabolites (MAA^{V}, $DMAA^{V}$) on day 1 and week 4

Arsenic species	Day		T_{max} (h)	C_{max} (ng/ml)	$t_{1/2,\beta}$ (h^{-1})	$AUC_{0-tlast}$ (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)	Vz (l/kg)	CLtot (l/kg/h)	Vss (l/kg)
Inorganic Arsenic[a] As^{III}+As^{V}	Day 1	Mean	1.9	22.6	15.4	138.6	211.8	15.2	0.7	14.3
		SD	0.7	11.4	9.2	32.4	55.1	6.7	0.2	6.3
	Week 4	Mean	2.0	23.2	24.2	233.3	474.8	12.8	0.8	12.5
		SD	0.3	10.2	12.5	92.8	192.6	10.6	0.5	10.4
As^{IIIa}	Day 1	Mean	1.9	12.4	17.0	52.8	80.5	44.0	2.3	55.9
		SD	0.7	8.4	19.0	21.2	39.8	27.5	1.3	55.9
	Week 4	Mean	1.6	10.1	24.8	82.6	190.6	41.5	2.7	39.7
		SD	0.5	6.6	12.3	55.7	189.9	28.6	1.7	28.1
As^{Vb}	Day 1	Mean	1.8	10.2	18.3	86.9	155.1	25.8	1.2	24.8
		SD	0.9	3.9	11.3	22.5	78.6	9.4	0.6	8.9
	Week 4	Mean	2.0	14.2	32.2	150.7	357.5	21.0	1.2	20.6
		SD	0.3	6.6	24.2	51.9	164.7	18.6	0.8	18.4
MAA^{Vc}	Day 1	Mean	18.0	3.1	–	48.7	–	–	–	–
		SD	6.4	1.6	–	12.9	–	–	–	–
	Week 4	Mean	3.9	10.9	–	174.2	–	–	–	–
		SD	6.5	4.7	–	66.1	–	–	–	–
$DMAA^{Vd}$	Day 1	Mean	20.1	5.4	–	83.1	–	–	–	–
		SD	6.3	2.9	–	30.8	–	–	–	–
	Week 4	Mean	5.6	21.4	–	374.1	–	–	–	–
		SD	9.2	12.3	–	214.5	–	–	–	–

– Not analyzable

T_{max} time to maximum concentration, C_{max} maximum concentration, $t_{1/2,\beta}$ apparent terminal half-life, $AUC_{0-tlast}$ area under the curve from time zero to the final measurable time point (t_{last}), $AUC_{0-\infty}$ area under the curve from time zero to infinity, Vz volume of distribution at the terminal phase, CL_{tot} systemic clearance, Vss volume of distribution at steady-state

The values obtained during the induction and consolidation treatment were used for patient 7, while the values obtained during the consolidation treatment were used for patient 8

[a] The values obtained from ten patients (N = 11) on day 1 and six patients (N=6) in week 4 were analyzed

[b] The values obtained from 11 patients (N = 12) on day 1 and six patients (N=6) in week 4 were analyzed

[c] The C_{max} and T_{max} were obtained from 12 patients (N=13) and $AUC_{0-tlast}$ was obtained from 9 patients (N=10) on day 1 while these parameters were determined in six patients (N=6) in week 4

[d] The C_{max} and T_{max} were obtained from 11 patients (N=12) and $AUC_{0-tlast}$ was obtained from 9 patients (N=10) on day 1 while these parameters were determined in six patients (N=6) in week 4

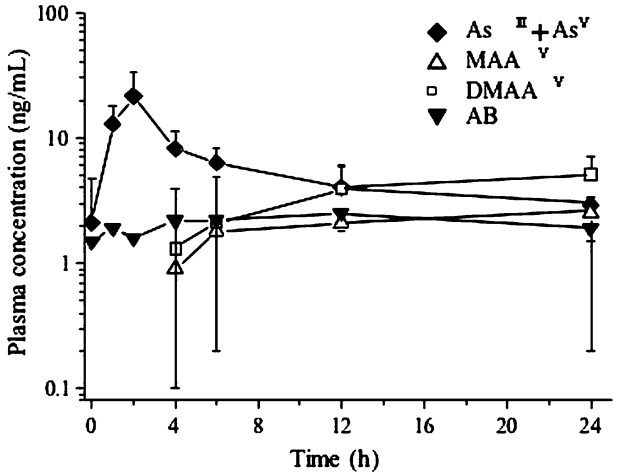

Fig. 2 Plasma concentrations of inorganic arsenic and methylated metabolites (MAA^{V}, $DMAA^{V}$, AB) on day 1 of the repeated administration. The values shown in the figure were determined in 12 patients (N = 14; mean ± standard deviation). The values obtained during the induction and consolidation treatment were used for patient 7 and 8

arsenic compound derived from seafood, remained almost constant (about 2 ng/ml) during the study period. Accordingly, the influence of arsenic derived from meals in this study was considered negligible.

For the 2 patients (patient 7 and 8) whose arsenic concentrations were determined both the induction and consolidation treatment, the plasma concentrations of As^{III}, As^{V}, and MAA^{V} had decreased below the quantitation limit during the washout period (69 days for patient 7; 37 days for patient 8) before the consolidation treatment, and only the $DMAA^{V}$ concentration of 1.9 ng/ml was detected in patient 8. For patient 7, the blood was collected 9 days after the completion of the induction treatment for 43 days receiving a total of 448 mg ATO, and the plasma concentrations were as follows: As^{V}—0, As^{III}—0, MAA^{V}—1.9 ng/ml, $DMAA^{V}$—8.3 ng/ml, indicating the complete disappearance of inorganic arsenic. These results indicate that most of the inorganic arsenic is

promptly metabolized to $DMAA^V$ and ATO is not accumulated in the blood.

Pharmacokinetics of arsenic species during the repeated administration

The mean plasma concentrations of inorganic arsenic ($As^{III} + As^V$) and its metabolites (MAA^V and $DMAA^V$) on day 1 and weeks 1, 2, and 4 during the repeated administration are shown in Fig. 3, and the pharmacokinetic parameters on day 1 and week 4 are in Table 2. In comparison with the levels on day 1, the C_{max} of inorganic arsenic on week 4 was similar but the elimination was delayed. As a result, the $AUC_{0-\infty}$ increased about twofold. However, the $AUC_{0-tlast}$ of inorganic arsenic on week 4 was similar to the $AUC_{0-\infty}$ on day 1, indicating that no marked change was observed in CL_{tot} during the repeated administration. The mean concentration profile from day 1 to week 4 indicated no increase in the C_{max} of inorganic arsenic related to administration frequency. These results suggest that the plasma concentration had reached the steady state. Alternatively, the plasma concentrations of MAA^V and $DMAA^V$ increased along with the increase in administration frequency during the repeated administration. The C_{max} and $AUC_{0-tlast}$ of both metabolites on week 4 were about four times those of the respective levels observed on day 1. The plasma concentrations of AB remained almost constant (about 2–4 ng/ml) during the repeated administration.

Urinary excretions of arsenic species

The urinary excretions (daily excretion rate, % of dose) of inorganic arsenic and methylated metabolites on day 1 and weeks 1, 2, and 4 during the repeated administration are shown in Table 3. The respective excretions of As^{III} and As^V on day 1 accounted for about 6%. During the repeated administration, the excretions increased and remained almost constant after week 1–4 (As^{III}: about 13–16%, As^V: about 7–8%), suggesting that the steady state was attained. A similar tendency was observed in the excretion rates of MAA^V and $DMAA^V$ on day 1 and during the repeated administration. The mean total arsenic excretion rate including inorganic arsenic and methylated arsenic was about 20% of daily dose on day1 and remained at about 60% of daily dose during week 1–4.

The CL_{re} values for As^{III} and As^V were about 10 and 6% of the CL_{tot} (shown in Table 2), respectively. These results indicate that renal excretion play no significant role in the elimination of inorganic arsenic, and suggest that hepatic elimination appears to be the main route of systemic clearance.

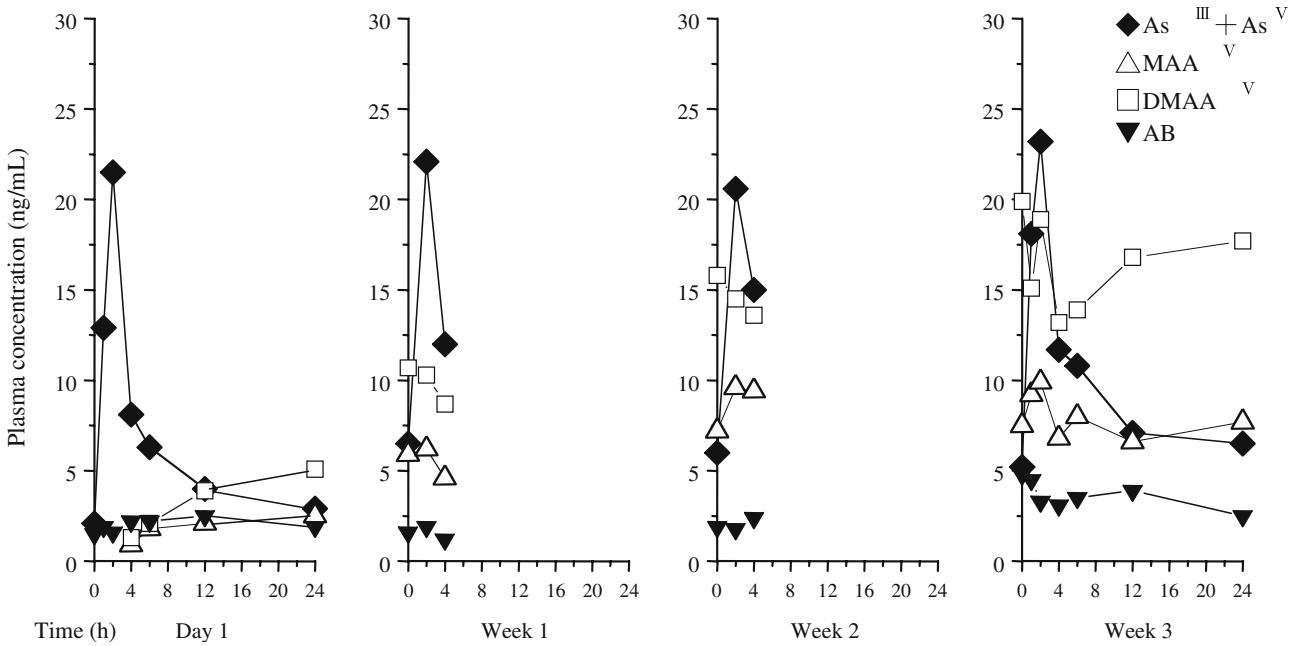

Fig. 3 The plasma concentrations of inorganic arsenic ($As^{III} + As^V$) and its metabolites (MAA^V, $DMAA^V$, and AB) on day 1 and weeks 1, 2, and 4. The values shown in the figure indicate mean values determined in 12 patients ($N = 14$) on day 1, in nine patients ($N = 10$) during week 1, in ten patients ($N = 12$) during week 2 and in seven patients ($N = 7$) during week 4. The values obtained in the induction and consolidation treatment were used for patients 7 and 8

Table 3 Urinary excretions of inorganic arsenic and methylated metabolites during the repeated administration

		Urinary excretions (% of dose)				CLre (l/kg/h)	
		Day 1	Week 1	Week 2	Week 4	Day 1	Week 4
As^{III}	Mean	6.5	16.2	12.8	13.7	0.20	0.32
	SD	4.9	10.1	8.3	9.6	0.10	0.14
As^{V}	Mean	5.6	7.3	8.2	6.8	0.07	0.07
	SD	5.9	8.6	12.1	8.2	0.06	0.06
MAA^{V}	Mean	5.0	17.4	12.9	19.6	0.11	0.17
	SD	2.5	11.2	5.9	10.0	0.07	0.10
$DMAA^{V}$	Mean	3.2	19.4	19.8	21.1	0.05	0.10
	SD	1.3	8.5	9.6	9.5	0.03	0.03
Total	Mean	20.4	60.3	53.7	61.1	–	–
	SD	7.4	25.1	22.6	28.5	–	–

The urine was collected for 24 h on each measurement day. The values obtained during the induction and consolidation treatment were used for patient 7 and 8. The urine samples obtained from nine patients ($N = 11$) on day 1, from nine patients ($N = 10$) during week 1, from nine patients ($N = 11$) during week 2 and from six patients ($N = 6$) during week 4 were analyzed

CL_{re} values were obtained from five to seven patients ($N = 6$–8) on day 1 and from four to five patients ($N = 4$–5) on week 4

Discussion

The plasma and urine concentrations of inorganic arsenic and methylated metabolites in APL patients treated with ATO were determined with HPLC/ICP–MS to clarify the pharmacokinetics of arsenic species. Until recently, arsenic was determined as the total arsenic content including metabolites in most of the reports on the pharmacokinetics of ATO. The current report therefore has considerable clinical meaning because it is the first study investigating the pharmacokinetics of arsenic species in as many as 12 Japanese patients with APL.

Arsenic is contained in foods and especially abundant in fish, shellfish, and seaweed. Arsenic compounds abundant in seafood are mainly organic such as arsenobetaine (AB), a trimethylarsenic compound. The toxicity of AB is extremely low and it is quickly excreted unmetabolized [17, 18]. Since the Japanese people ingest a large quantity of seafood, their urinary concentration of arsenic is higher as an ethnic characteristic. Then the influence of AB derived from meals cannot be ignored, especially for the Japanese. It is possible to determine AB separately from other arsenic compounds in plasma and urine by HPLC/ICP-MS. Therefore this method is very effective to analyze the pharmacokinetics of arsenic species in the Japanese APL patients.

In our study, As^{V} was detected in the plasma and urine at a concentration equivalent to or higher than that of As^{III} and the pharmacokinetic parameters of inorganic arsenic (As^{III} + As^{V}) were similar to those of As^{III} in the Westeners. [Remick et al. J Clin Oncol 2004; 22:2018 (abstract)]. The previous reports have shown that the As^{V} concentrations in plasma or urine after ATO administration are very low. [15, 16, 19] The conversion from As^{III} to As^{V} occurs as natural oxidation while arsenate reductase mainly contributes to the reduction from As^{V} to As^{III} [20]. In general, the pentavalent arsenic compound is more stable than the trivalent arsenic compound. Feldmann et al. [21] reported that As^{III}, As^{V}, MAA^{V}, and $DMAA^{V}$ remained stable up to 2 months in human urine at 4°C or –20°C, but that about 30% of As^{III} was oxidized to As^{V} in some urine samples after storage at 4°C for longer than 4 months. Del Razo et al. [22] analyzed the stability of arsenic compound in aqueous solution and urine at 4°C and reported that 20 and 21–32% of As^{III} were oxidized, respectively after 2 months. Considering that the plasma and urine samples analyzed in our study were stored frozen for about 5 years, it is highly likely that the As^{V} in the plasma and urine was generated by oxidation of As^{III} during the storage period.

During the repeated administration, the plasma concentrations of inorganic arsenic reached the steady state, whereas accumulation of MAA^{V} and $DMAA^{V}$ in the blood was related to administration frequency. These accumulations were observed also in the Westerners. The total arsenic concentrations reported previously [11, 12, 14] indicated a tendency to increase during the repeated administration of ATO to patients with APL and other hematological malignancies. The results obtained in our study, indicate most of the increase in the total arsenic concentrations can be attributed to the accumulation of methylated metabolites.

During the repeated administration, approximately 60% of the administered arsenic trioxide dose was

excreted in urine as inorganic arsenic and methylated species in Japanese patients. There was not marked difference in the urinary excretions of inorganic arsenic ($As^{III} + As^{V}$) between the Westerners and the Japanese. These results were similar to those in the reports for the excretion of arsenic in humans [15, 23, 24]. In addition, CL_{re} was much lower than CL_{tot} for inorganic arsenic, indicating that renal excretion plays no significant role in the elimination of inorganic arsenic. It is therefore considered that the plasma concentrations of inorganic arsenic do not increase in patients with impaired renal function, as shown by Remick et al. [J Clin Oncol 2004; 22:2018 (abstract)] These results suggest that there were no marked differences in the plasma profiles and urinary excretions of inorganic arsenic and methylated metabolites during the repeated administration of ATO between the Westerners and the Japanese APL patients.

The problem that should be considered when treating APL patients with ATO is chronic arsenic poisoning which is induced by the intratracheal and oral exposure to a comparatively small amount of arsenic for a long term (ten or more years). The symptoms demonstrated in chronic arsenic poisoning are disorders in the skin, mucosa, peripheral nerves, liver, and respiratory organs as well as skin and lung cancer. According to the previous reports, the total arsenic concentrations in the blood of inhabitants who drank well water contaminated with arsenic (5–410 ng/ml) were 2–42.1 ng/ml [25]. These results are similar to the plasma concentrations in this study. Though the liver function abnormalities and peripheral neuropathies occurred in patients of this study [6], they have improved after completion of administration. In the case of treatment for APL, the administration of ATO is not continued permanently or long-term and arsenic is promptly excreted during the washout period. Therefore, it is unlikely that the treatment with ATO results in chronic arsenic poisoning, although sufficient attention should be paid to the adverse reactions during the treatment.

In conclusion, the present study showed the pharmacokinetics of arsenic species in Japanese APL patients treated with ATO. The plasma concentrations of inorganic arsenic (As^{III} and As^{V}) reached the C_{max} immediately after completion of administration followed by a biphasic elimination. However, the appearance of methylated metabolites (MAA^{V}, $DMAA^{V}$) in the blood was delayed. During the repeated administration, the pharmacokinetics of inorganic arsenic reached the steady state but the concentrations of MAA^{V} and $DMAA^{V}$ increased in relation to administration frequency. ATO is metabolized when administered intravenously to APL patients and methylated metabolites are promptly eliminated from the blood and excreted into urine after completion of administration, consequently indicating no measurable accumulation of ATO in the blood. These results are considered important information for the current and future clinical use of ATO.

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Appendix

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