Clinical Pharmacokinetic Study of Arsenic Trioxide in an Acute Promyelocytic Leukemia (APL) Patient: Speciation of Arsenic Metabolites in Serum and Urine

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The pharmacokinetics of arsenic species in a Japanese patient with relapsed acute promyelocytic leukemia (APL) treated with arsenic trioxide at a daily dose of 0.08 mg/kg was investigated. After achieving complete remission on Day 35 during the induction therapy of arsenic trioxide, we collected the serum and urine samples on Days 4 and 5 during the consolidation therapy of arsenic trioxide. The concentrations of inorganic arsenic and the methylated metabolites in serum and urine were measured by HPLC/ICP-MS. The patient restricted the seafood for 3 d before the start of administration and during the sampling period in order to avoid the influence of arsenic derived from seafood. Arsenite (AsIII), methylarsenic acid (MMAs V), and dimethylarsinic acid (DMAs I) were detected in serum and urine. The total concentration of AsIII, MMAs V and DMAs I in serum ranged from 18 to 41 μg/l (240–547 nM) during 24 h on Day 4. The amount of total arsenic (AsIII+MMAs V+DMAs I) in urine was 4464 μg/d on Day 4. These results suggest that not the micro-molar but the nano-molar order of arsenic in serum is sufficient to produce the therapeutic effect on APL cells.

Key words acute promyelocytic leukemia (APL); arsenic trioxide; arsenic metabolite; serum concentration; urinary excretion

Acute promyelocytic leukemia (APL) is characterized M3 in the French–American and British (FAB) classification and represents approximately 5—10% of all leukemia in adults.1) In about 95—98% of the cases, APL is associated with the reciprocal translocation, t(15; 17)(q22; q21)2,3) and the reciprocal fusion of the retinoic acid receptor (RAR)α gene on chromosome 17 with the promyelocytic leukemia (PML) gene on chromosome 15. The resulting PML-RARα fusion gene encodes a chimeric protein4,5) that causes the pathogenesis of APL.2,6)

Huang et al. reported that all-trans retinoic acid (ATRA)—a vitamin A derivative—yielded a high rate of complete remission for APL patients.7) Several clinical studies have shown that ATRA treatment in combination with chemotherapy induced survival rate of 67—84% at 2—4 years.8—11) Currently, ATRA has become a first-line treatment of newly diagnosed APL.8—11) The combination of ATRA with chemotherapy can obtain long-term survival in 70% of newly diagnosed APL. Nevertheless, the remaining 30% of patients relapse and are often resistant to further treatment with ATRA and chemotherapy.12)

In 1996, the Chinese Shanghai II Medical University Group reported that arsenic trioxide induced apoptosis of APL cells,13) and 14 of 15 patients who relapsed after ATRA therapy achieved complete remission (CR) with no serious adverse events.14) Several studies reported that intravenous infusion of arsenic trioxide was effective in approximately 90% of relapsed APL patients who are resistant to ATRA and chemotherapy.14—16) However these studies have not accurately investigated the metabolism of arsenic trioxide after the intravenous infusion.

In this study, arsenic trioxide was administered intravenously to ATRA-resistant APL patient, and we determined the efficacy of arsenic trioxide in APL as well as the serum or urine concentrations of inorganic arsenic and the methylated metabolites.

MATERIALS AND METHODS

Patient The patient was a 72-year-old male patient weighing 57.5 kg. In September 1999, he was diagnosed with APL. The marrow smears showed increased promyelocytes (80%). Chromosome analysis revealed 47; X, Y, t(15; 17)(q22; q21)k, +8, t(15; 17)(q22; q11—21). He achieved first CR with ATRA and chemotherapy. Subsequently the consolidation therapy was started on Day 56, which led to PML-RARα negative, followed by one-year maintenance therapy with ATRA. In January 2001, He relapsed and achieved second CR with ATRA. However, in July 2001, the bone marrow smear obtained during the maintenance therapy revealed an increase of promyelocytes, and the patient was diagnosed as relapsed and ATRA resistant APL. Because the cumulative dose of anthracyclines had reached a limiting dose, and the patient was elderly, the induction therapy with further chemotherapy seemed to be highly risky. Before arsenic trioxide treatment, the protocol was reviewed and approved by the institutional ethical committee of our hospital, and written informed consent was obtained from the patient and his family (based on the ethical principles of the Declaration of Helsinki).

Evaluation of Treatment Efficacy We evaluated the efficacy of arsenic trioxide by monitoring the number of leukemic cells of bone marrow collected at 6 d before treat-
ment and 7, 8, 35, 41 and 54 d after the start of treatment. Bone marrow cells were obtained by aspiration of an iliac bone. The smears were stained with May–Giemsa’s solution and differential counts were evaluated on 500 nucleated cells.

**Arsenic Trioxide Preparation** A 0.1% arsenic trioxide solution for injection was prepared in the pharmacy of Nagano Red Cross Hospital. For this preparation, we purchased arsenic trioxide (98% As$_2$O$_3$ reagent) and other reagents from Wako Pure Chemical Industries, Ltd. The solution was prepared as follows:

Arsenic trioxide 0.2 g was weighed and placed in a stoppered measuring cylinder. Approximately 140 ml of distilled water for injection was added, and then 19 ml of 1 N sodium hydroxide (special grade) was added to completely dissolve arsenic trioxide. Subsequently, hydrochloric acid (special grade) was added to adjust the pH of the solution to pH 5—6. The remaining distilled water for injection was added to make a total volume of 200 ml. The solution was then divided into measured quantities of 10 ml each, filtered into sterile vials (using a 0.22 μm filter) and was sterilized in an autoclave. The solution required storage in lightproof, air-tight containers at room temperature.

For intravenous injection to the patient, arsenic solution equivalent to 4.6 mg/d (3.5 mg of arsenic) was added to 5% glucose solution (Kobayashi Pharmaceutical Industry Co., Ltd.), and the resulting solution was continuously infused by IV drip over a 2-h period each treatment day.

**Therapy Protocol and Sample Collection for Pharmacokinetic Study** Arsenic trioxide was administered intravenously daily over 2 h at a dose of 0.08 mg/kg for 35 consecutive days during the induction therapy, and for 28 consecutive days during the consolidation therapy.$^{17}$ The interval between the induction and the consolidation therapy was 55 d.

For pharmacokinetic analysis, serum samples were collected at 11 time points and 24-h urine samples were collected on days 4 and 5 during the consolidation therapy. The time points for blood collection (1—2 ml) were as follows. Immediately before administration, 2 h (at the end of administration), 3, 4, 5, 6, 7, 8, 10, 19 and 24 h after administration. After the urine sample collection, the urine volume was measured.

**Arsenic Determination** The quantification of arsenic compounds was performed by HPLC/ICP-MS, which combines high-performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS).$^{18,19}$

As the standard arsenic compounds, sodium arsenite, sodium arsenate, sodium methylarsonate (MMAs), dimethylarsinic acid (DMAs), trimethylarsine oxide (TMAOs), arsenobetaine (AsBe), arsenocholine (AsCho) and tetramethyl arsonium (TetMAs) were purchased from Tri Chemical Laboratories Inc. Inertsil AS (150 mm×2.1 mm, 3.0 μm) was used to isolate the arsenic compounds and an ODS guard column was attached to allow direct injection of the biological samples. Inertsil AS and ODS guard column were purchased from GL Sciences Inc. A mobile phase was prepared using 10 mM butane sulfonic sodium (special grade), 4 mM malonic acid(special grade) and 4 mM tetramethylammonium hydroxide (special grade), and adjusted to pH 3.0 with HNO$_3$. A flow rate of mobile phase was 0.2 ml/min. To the serum samples, the same volume of acetonitrile was added for deproteinization, and after filtration of the supernatant fluid, the same volume of mobile phase was added for dilution. Butane sulfonic sodium, malonic acid, tetramethylammonium hydroxide and acetonitrile were purchased from Wako Pure Chemical Industries, Ltd. To the urine samples, the same volume of mobile phase was added for dilution, and filtered to obtain the solution for injection.

The analytical condition of ICP-MS was as follows, argon was used for plasma gas and flow rate was 181/min, ICP RF power was used at 1500 W and also arsenic was monitored at m/z 75. Five microliters of sample solution was injected onto the guard column and the amount of each arsenic compound was obtained from the calibration curve.

**Restriction of Seafood** Arsenic occurs in various foods and particularly in seafood. Some foods contain arsenic exceeding 100 μg/g.$^{20}$ Arsenic contained in fish or shellfish is mainly organic arsenic such as AsBe and trimethylarsenic compound, whereas arsenic contained in seaweed consists of dimethylarsenic compound and inorganic arsenic.$^{20}$ Therefore, considering the biological half-life of arsenic, we restricted the patient taking meal with seafood for 3 d before the start of administration and during the sampling period in order to accurately evaluate the pharmacokinetics of arsenic metabolites.

**Definition of Total Arsenic** Most of the inorganic arsenic (As$^{III}$/As$^{V}$) in mammals undergoes metabolic conversion in the liver, to MMAs and DMAs, and partially trimethylated metabolites (Fig. 1).$^{21}$ However, arsenic is mainly metabolized to DMAs$^{V}$ in humans,$^{22}$ and hardly metabolized to trimethylated metabolites. We therefore defined total arsenic as the sum of arsenate (As$^{V}$), arsenite (As$^{III}$), MMAs$^{V}$ and DMAs$^{V}$.

**RESULTS**

**Treatment Efficacy** Arsenic trioxide therapy was started in July 19, 2001. The count and percentage of promyelocyte were $32.6\times 10^9/\mu l$ and 90.6% on Day 7 and $0.2\times 10^9/\mu l$ and 0.6% on Day 35 during the induction therapy. The patient achieved complete remission, and arsenic trioxide was discontinued. On Day 16 during the therapy, APL syndrome-like rapid leukocytosis and fever were observed, which improved with additional chemotherapy (Ara-C) and steroids.
The additional administration of Ara-C could suppress rapidly leukocytosis. The consolidation therapy of arsenic trioxide for 28 d was started in October 17. The patient received dalteparin Na to prevent bleeding due to disseminated intravascular coagulation (DIC), and required blood transfusion of fresh frozen plasma (FFP) and platelet (PLT) nearly every day. The timeline of drug administration and collection of clinical data during the induction therapy are shown in Fig. 2.

**Adverse Events**

The common adverse events that have been reported with arsenic trioxide therapy include ventricular arrhythmia and QT prolongation. Shen et al. reported that the incidence of cardiotoxicity, digestive tract symptoms and hepatic dysfunction decreased at the low dose (0.08 mg/kg) as compared with conventional dose (0.15 mg/kg), but the therapeutic effect was observed at both doses. Zhou et al. reported that 35—52.5% of APL patients who received arsenic trioxide treatment developed tachycardia and QT prolongation within 1 to 2 weeks after the start of therapy.

The adverse events observed in our study included headache, diarrhea, dermatitis, premature ventricular contraction (PVC) and QT prolongation. Headache, diarrhea and dermatitis were continuously present during the therapy. PVC developed at 2 weeks after the start of therapy and improved by continuous intravenous infusion of lidocaine. QTc was prolonged from 430 to 466 ms. However, these adverse events were not serious and could be treated with symptomatic therapy.

**Concentrations of Inorganic Arsenic and the Methylated Metabolites in Serum and Urine**

The typical chromatograms of serum and urine by HPLC/ICP-MS are shown in Figs. 3 and 4. As$^{III}$, MMA$^V$ and DMA$^V$ were detected in serum and urine. Also, an unknown arsenic peak was observed in serum samples. No AsBe was detected in either serum or urine.

The serum concentration profiles of As$^{III}$, MMA$^V$ and DMA$^V$ on Day 4—5 during the consolidation therapy are shown in Fig. 5. The concentrations of As$^{III}$, MMA$^V$ and DMA$^V$ were 9.1, 8.2 and 10.4 μg/l (121, 109, and 139 nM) respectively before the administration and increased to 13.1, 13.2 and 14.4 μg/l (175, 176, and 192 nM) respectively at 2 h after the start of administration, namely immediately after the completion of administration. However, they decreased gradually from the completion of administration and remained at almost constant level for up to 24 h after the start of administration. The maximum concentration of total arsenic observed immediately after the completion of administration was 40.7 μg/l (543 nM), and the trough value at 24 h after the start of administration was 18.2 μg/l (243 nM). The concentration ranges were 5.8—13.1 μg/l (77—175 nM) for As$^{III}$, 5.5—13.2 μg/l (73—176 nM) for MMA$^V$ and 5.1—14.4 μg/l (68—192 nM) for DMA$^V$.

In the urine sample (1200 ml) collected for 24 h after the administration of arsenic trioxide (3.5 mg As), the arsenic concentrations were 840 μg/l (11.2 μM) for As$^{III}$, 1570 μg/l (20.9 μM) for MMA$^V$ and 1310 μg/l (17.5 μM) for DMA$^V$. The total amount of inorganic arsenic and the methylated metabolites was 127.5% of the daily dose.

**DISCUSSION**

**Efficacy of Arsenic Therapy**

Previous reports indicate that arsenic trioxide does not have the immediate effect during the induction therapy. Shen et al. reported that the median time to achieve CR was 31 d in 26 patients, which was comparable to our results (28 d after the start of administration). Therefore, in order to produce the therapeutic effect on
APL cell, it is an important factor to maintain blood level of arsenic to induce apoptosis and differentiation of APL cells in a time-dependent manner.

**Analytical Data**  Until recently, arsenic was determined as the total arsenic content in most reports involving arsenic trioxide therapy. Although several studies have reported the pharmacokinetics of the arsenic species including the metabolites, these reports have not considered the influence of arsenic derived from seafood. Since the Japanese people ingest a large quantity of seafood, the urinary concentration of arsenic is higher as an ethnic characteristic; mean = 130 μg/l (1.7 μM) for the ethnic groups that commonly ingest seafood (like the Japanese)\(^25,26\) and below 10 μg/l (133 nM) for the ethnic groups that ingest little seafood, respectively.\(^27\)

The individual differences and day-to-day fluctuations on the serum or urinary concentrations of arsenic also are remarkable in the Japanese.\(^20,28\) We therefore restricted the patient taking a seafood-derived diet to exclude the effect of arsenic derived from seafood and confirm the pharmacokinetics of arsenic trioxide for APL therapy.

The total arsenic excretion rate including inorganic arsenic and the methylated metabolites in our study was 127.5% of the daily dose suggesting that the arsenic compounds were accumulated in the body during the consecutive administration.

Fig. 3. Chromatogram of Patient Sample (Serum) for Analysis of HPLC/ICP-MS

75ArCl ion (19Ar + 13Cl) peak at m/z 75, caused from argon plasma and chlorine ion in biological samples as human urine or serum, interfered on the chromatograms near arsenate ion peak. The 75ArCl ion is making a mistake in a peak on chromatograms, and requiring cautions.

Fig. 4. Chromatogram of Patient Sample (Urine×100) for Analysis of HPLC/ICP-MS

In the general Japanese people, AsBe derived from seafood are detected in urine. Since we restricted the patient taking meal with seafood, AsBe were not detected in urine, suggesting that the urinary concentration of arsenic compounds represents that of the administered arsenic trioxide for APL therapy.
In our study, the urinary excretion rate of total arsenic was high as compared with the previous reports; 1—8% by Shen et al.\textsuperscript{14} and 6.1—31.0% by Westervelt et al. (mean = 18.7%).\textsuperscript{30}

The composition of urinary arsenic metabolites in the general Japanese population who have not been exposed to arsenic trioxide for the purpose of medical treatment are approximately 60% for TMAs such as AsBe, approximately 30% for DMAs, 3—5% for MMAs and 5—10% for inorganic arsenic.\textsuperscript{20,27} Recently, Suzuki reported that the major metabolites and excrements of arsenic are DMAs.\textsuperscript{22}

However, our results showed the urinary excretion of MMAs was high (42.2%) as compared with these data. This may be associated with that the activity of arsenic-metabolizing enzymes and the amount of S-adenosylmethionine (SAM) and glutathione (GSH) in the liver decreases during the consecutive administration, leading to the presence of a large amount of MMAs in blood, incomplete metabolism to DMAs and excretion of MMAs in urine. It appeared that consecutive administration of arsenic trioxide was responsible for the increase of the urinary excretion of MMAs.

Considering the timing of morphological CR and accumulation of MMAs in serum and urine, the appearance of MMAs may be an important factor in the treatment of APL.

This study clarified that the concentration of the primary metabolite MMAs was high in serum and urine, and that administered arsenite (As\textsuperscript{III}) was methylated through hepatic metabolism, producing intermediate MMAs, and underwent further methylation to DMAs. The serum concentration of arsenic metabolites increased slightly after the completion of drip infusion (2 h after the start of administration), but the concentration was nearly constant for up to 24 h. The peak serum concentration of total arsenic was 40.7 µg/l (543 nm) and the trough level was 18.2 µg/l (243 nm). These concentrations were comparable with the blood arsenic concentration in Chinese patients with chronic arsenic poisoning as reported by Yoshida \textit{et al.}\textsuperscript{31} In most reports involving arsenic trioxide therapy, blood arsenic concentrations were the order of the micro-molar.\textsuperscript{17,29} In contrast, our results showed the therapeutic effect of arsenic on APL cells at the order of nano-molar.

The administration method of a daily intravenous infusion over 2 h produces a concentration that does not cause acute arsenic poisoning and maintains blood level of arsenic in the order of nano-molar (µg/l), which is equivalent to concentrations in patients with chronic arsenic poisoning in China, etc.\textsuperscript{1} This administration method seems to be reasonable to create the appropriate environment to induce apoptosis or differentiation of APL cells.

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\textbf{REFERENCES AND NOTES}