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Pharmacokinetic of Arsenic Trioxide in Newly Diagnosed Acute Promyelocytic Leukemia Patients

[1]R. Hosseini, [1,4]A. Mandegary, [2,3]K. Alimoghaddam, [2]A. Ghavamzadeh and [1,3]M.H. Ghahremani
[1]Department of Toxicology and Pharmacology, Faculty of Pharmacy,
Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran
[2]Hematology, Oncology and BMT Research Center, Shariati Hospital, Tehran, Iran
[3]Department of Molecular Medicine, Faculty of Advanced Technology in Medicine,
Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Toxicology and Pharmacology, Faculty of Pharmacy,
Kerman University of Medical Sciences, Kerman, Iran

Abstract: The high complete remission rate with arsenic trioxide (ATO) in relapsed Acute Promyelocytic Leukemia (APL) patients has been led to its use in newly diagnosed patients. Twenty newly diagnosed APL patients between January 2006 and 2007 received 2 h intravenous infusion of 10 mg day^{-1} arsenic trioxide for induction therapy until achieving complete remission. Plasma arsenic concentration was analyzed by graphite furnace atomic absorption method by dilution of plasma with a suitable matrix modifier. The concentration of arsenic in 24 h urine of patients was measured by using a valid standard addition method and a suitable matrix modifier. The Limits of Detection (LOD) were 1.2 and 1.5 μg L^{-1} for arsenic in plasma and urine, respectively. Pharmacokinetic parameters of 20 patients were as following: C_{max}: 43.6 ±19.5 μg L^{-1}, t_{max}: 2.15±0.7 h, AUC_{0-24}: 683±317 μg h L^{-1}, $AUC_{0-\infty}$: 2027±958 μg h L^{-1}, $t_{1/2}$: 41±10 h, k_{el}: 0.02±0.01 h^{-1}, V_d: 5.6±3.6 L kg^{-1} and Cl_{total}: 0.1±0.05 L kg^{-1} h^{-1}. During the first day of induction, 1.4±0.2% of administrated arsenic excreted into urine. Renal clearance was 5.1±4.1 mL kg^{-1} h^{-1}. However, the results showed that the pharmacokinetic of ATO in newly diagnosed APL patients weren't dependent to the sex of patients.

Key words: Arsenic trioxide, pharmacokinetic, newly diagnosed APL, Graphite furnace atomic absorption

INTRODUCTION

Acute Promyelocytic Leukemia (APL) is a well-defined subtype of leukemia accounts for 10-15% of acute myeloid leukemias. APL is characterized by PML/RARα fusion protein resulted of a reciprocal translocation between the retinoic acid receptor-α (RARα) gene on the chromosome 17 with the PML gene on the chromosome 15. The PML/RAR-α is leukemogenic and blocks differentiation of promyelocytes. The disease is associated with a potentially life-threatening hemorrhage diathesis which has been attributed to Disseminated Intravascular Coagulation (DIC) (Grignani *et al*., 1994; Barbui *et al*., 1998). Before 1992, induction therapy for patients with APL was similar to all other patients with Acute Myeloid Leukemia (AML) and included an anthracycline and cytarabine (Tallman *et al*., 2002). After introduction of All Trans Retinoic Acid (ATRA) as the first paradigm of differentiation therapy, APL that was once characterized by a high early death rate due to fatal bleeding became the best curable subtype of Acute Myeloid Leukemia (AML) (Lengfelder *et al*., 2005). Despite excellent initial responses, Complete Remission (CR) of 90%, APL cells develop resistance to ATRA and relapse occurs in one-third of the patients who have already obtained CR within 5 years (Warrell *et al*., 1991; Grignani *et al*., 1994; Tallman *et al*., 2002). Recently, arsenic compounds, mainly arsenic trioxide (ATO) have been used in primary APL patients as well as in relapsed cases. In most studies, the ATO dosage has been 10 or 0.15 mg kg^{-1} day^{-1} until achieving CR or maximum for 60 days. In the molecular level, arsenic trioxide exerts dual effect on APL cells: induction of apoptosis (1-2 μmol L^{-1}) and differentiation (0.1-0.5 μmol L^{-1}). There are evidences that pharmacokinetic parameters of ATO in blood have a direct relation to its dosage. For example, it is shown that low-dose ATO had the same therapeutic effect as the

Corresponding Author: R. Hosseini, Department of Toxicology and Pharmacology, Faculty of Pharmacy,
Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran
Tel: +9821 6695 9103 Fax: +9821 6643 7751

conventional dosage and even lower toxicity (Shen *et al*., 2001). The pharmacokinetic of ATO has been done in several studies (Shen *et al*., 1997, 2001; Wang *et al*., 2004; Fukai *et al*., 2006) but in spite of using of ATO in Iran for several years, so far no study has been done to determine the pharmacokinetic parameters of this compound. Therefore, evaluating the pharmacokinetic parameters of ATO and their changes according to dosage in the APL patients could shed lights on understanding the mechanisms involve in ATO therapy and to improve the decrease of adverse effects. So, we studied the pharmacokinetic of ATO injection in 20 newly diagnosed APL patients, by measurement of total arsenic concentration in plasma and urine samples using valid Graphite Furnace Atomic Absorption Spectrophotometric (GFAAS) methods (Nixon *et al*., 1991; Campillo *et al*., 2000) with some modifications.

MATERIALS AND METHODS

Twenty patients with newly diagnosed APL received induction therapy with arsenic trioxide (manufactured by Sina Darou, Tehran, Iran) between January, 2006 and 2007. Patients eligible for study participation were newly diagnosed APL patients with age between 10-75 years and with normal function of vital organs. Patients with CNS hemorrhage, age older than 75 years, history of hepatitis or hepatic failure (Bilirubin >50 mg L^{-1}), renal failure (S.Cr >20 mg L^{-1}) and heart failure were excluded from the study. The diagnosis of APL was established according to clinical presentation and morphological criteria of the French-American-British (FAB) classification and was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for PML/RARα transcripts as described recently Ghavamzadeh *et al*. (2006). All the patients had the presence of this transcript. Blood samples were taken from patients after informed consent, in accordance with regulations and protocols sanctioned by the Human Subjects Committee of Tehran University of Medical Sciences (TUMS) from the patients or their legal representative.

Treatment protocol

Induction therapy: Patients received ATO immediately after diagnosis as a 2 h intravenous infusion of 10 mg day^{-1} in 500 mL dextrose water until achievement of Complete Remission (CR) or maximum of 60 days. The patients did not receive any chemotherapeutic agents during the course of treatment. Consolidation therapy; Twenty-eight days after completion of induction therapy, consolidation therapy was started. It consisted of outpatient infusion of ATO at dose of 10 mg day^{-1}, 6 days a week for a total of 28 infusions. During this period, patients visited every week and Complete Blood Count (CBC), liver enzymes, renal function and electrocardiogram were evaluated.

Reagents and standards: Nitric and hydrochloric acids and $Ni(NO_3)_2$ were obtained from Merck, Germany. Tween-20 surfactant and stock arsenic standard solution (1000 ppm) were obtained from Sigma.

Sample collection and storage: In the first day of induction therapy, patients received 10 mg ATO in 500 mL dextrose water. At the times of 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after infusion, venous blood samples were collected into acid washed arsenic-free glass tubes which contain 70 μL heparin as anticoagulant and the tubes were centrifuged at 2500 g and separated plasma samples were stored at -70°C until analysis. Furthermore, 24 h urine was collected during the first day of infusion in acid washed containers without any additive. After measuring the 24 h urine volume, two 10 mL aliquot of urine stored at -70°C until analysis.

Sample preparation: Before the measurement of arsenic in plasma and urine samples by graphite furnace atomic absorption technique, the samples were treated with the suitable matrix modifiers. Therefore, various dilution ratios and concentrations of reagents were tested before we adopted the following preparation procedure, which yielded the most reproducible results.

Plasma: Plasma samples were diluted two folds by mixing one volume of plasma with one volumes of matrix modifier [2 g L^{-1} of Ni $(NO_3)_2$ and 10 g L^{-1} Triton X-100] and 20 μL of the final mixture was injected onto a pyrolytically coated graphite tube for analysis.

Urine: Urine specimens were diluted 1:1 (v/v) ratio with an aqueous solution containing 0.4% (w/v) nickel as Ni $(NO_3)_2$, 0.2% (w/v) Triton X-100, 10% (w/v) H_2O_2 and 1% (v/v) of 65% HNO_3 as a matrix modifier. We injected 20 μL of this solution onto a pyrolytically coated graphite tube for analysis. Present results showed that calibration of instrument using aqueous standard was not suitable to measure arsenic in urine. Therefore, the standard addition method was used to measure arsenic concentration in urine samples.

Instrument parameters

Equipment: We used an atomic absorption spectrophotometer model AA-680 (Shimadzu, Japan) and a model GFA-4B graphite furnace atomizer (Shimadzu,

Japan) with a deuterium continuum background corrector. The Shimadzu hollow-cathode lamp conditions for arsenic were working current of 6 mA, at 193.7 nm and 0.6 nm slit-width. We used pyrolytically coated graphite tubes (Shimadzu, Japan) throughout the study. Because reproducible drying of biological samples can be difficult, careful ramping during this stage is necessary. We optimized our temperature program and the final furnace conditions are shown in Table 1. Argon was used as the carrier gas. We routinely pro-conditioned the graphite tube with several injections of matrix modifier.

Pharmacokinetic parameters and statistical analysis: Peak plasma arsenic concentration (C_{max}) and time to reach peak concentration (t_{max}) for first day of ATO infusion were calculated from actual plasma data of each patient. Area under the plasma concentration-time curve (AUC) calculated as follow: the AUC up to the final measurable time point (t_{last}) was calculated by the trapezoidal method ($AUC_{0-tlast}$) and added to Ct_{last}/k_{el} to calculate $AUC_{0-\infty}$, where Ct_{last} is the concentration at t_{last}. Elimination rate constant (K_{el}) of arsenic was calculated by using the slope of curve and the formula of k_{el} = -Slope×2.303. Total clearance (Cl_{tot}) was calculated as dose/$AUC_{0-\infty}$ on day 1. Volume of distribution (Vd) was calculated from dose/(k_{el}×$AUC_{0-\infty}$) formula. Finally elimination half life ($t_{1/2}$) was calculated by using the formula $t_{1/2}$ = 0.693/K_{el}. On the basis of urinary concentrations, the urinary excretion (% of dose) and the renal clearance (Cl_{re}) were calculated from the following equations:

$$\text{Urinery excretion (\%)} = \frac{\text{Urinery concentration } (\mu g\,mL^{-1}) \times \text{Total volume } (mL^{-1})}{\text{Dose } (\mu g \times body^{-1})}$$

$$\text{Renal clearance } (L \times kg\,h^{-1}) = \frac{\text{Urinery excretion } (\mu g) \,/\, \text{body weight } (kg)}{AUC\,(\mu g \times h\,L^{-1})}$$

The data analyzed using SPSS.11. Independent student's t-test was used to find significant differences between two groups. $p<0.05$ was taken to be statistically significant.

Table 1: Optimized instrumental parameters (a) and temperature program for measurement of arsenic in plasma (b) and (c) urine by GF-AAS method

Variable	Optimized value
Wavelength	193.7 nm
Hallow cathode current	6 mA
Slit width	Plasma sample: 0.6 nm Urine sample: 0.8 nm
Tube type	Pyrolytically coated graphite tube
Background correction method	D_2 lamp
Measurement mode	Peak area
Sample volume	20 μL

Temp. (°C)	Time (sec)	Rate	GFR (L min^{-1})
Plasma			
150	40	Ramp	1.5
150	30	Step	1.5
700	25	Ramp	1.5
700	20	Step	1.5
700	4	Step	0.0
2400	4	Step	0.0
2600	4	Step	1.5
20	40	Step	1.5
Urine			
120	15	Ramp	1.5
120	30	Step	1.5
1400	20	Ramp	1.5
1400	20	Step	1.5
1400	4	Step	0.0
2400	4	Step	0.0
2500	4	Step	1.5
20	40	Step	1.5

Temp.: Temperature, GFR: Gas Flow Rate, GF-AAS: Graphite-Furnace Atomic Absorption Spectrophotometry

RESULTS

The demographic features and pharmacokinetic parameters of each patient are shown in Table 2. At the end of induction therapy with ATO in the twenty newly diagnosed APL patients, seventeen patients (85%) achieved a complete hematological remission. Two patients died of respiratory problems after APL-maturation syndrome in the early 10 days of treatment. One patient discontinued ATO administration on day 22 because of renal problems and constant increment in the creatinin and BUN levels in the blood. In this patient ATO therapy was displaced by chemotherapy (ATRA+Idaurobicin) until the end of induction. Of 20 patients nine were male (45%) and eleven (55%) were female. The means of the weight and age of all patients were 69.3 kg (range: 45-115 kg) and 31.4 years (range: 15-55 years). The results showed that plasma concentration of arsenic reached the peak level with mean C_{max} 43.6 μg L^{-1} rapidly (t_{max}: 2.15±0.75 h) after the administration in most of the patients, followed by a slow decline with a mean $t_{1/2}$ of 41±10 h (Table 2, Fig. 1). The mean volume of distribution (Vd) was large (5.6 L kg^{-1}) suggesting extensive distribution throughout the body. The other pharmacokinetic parameters of the patients were as following: AUC_{0-24}: 683 μg h L^{-1} (range, 233-1177 μg h L^{-1}), $AUC_{0-\infty}$: 2027 μg h L^{-1} (range, 686-4123 μg h L^{-1}), k_{el}: 0.02 h^{-1} (range, 0.01–0.03 h^{-1}) and Cl_{total}: 0.09 L kg^{-1} h^{-1} (range, 0.01-0.26 L kg^{-1} h^{-1}) (Fig. 1). After dividing amount of ATO to the weight, the dose of ATO obtained for each patient. Figure 2 shows the change of total arsenic concentration in plasma according to the dose of ATO. As the usual dosage of ATO is 0.15 mg kg^{-1} day^{-1}, we categorized the patients to 2 groups of dose <0.15 mg kg^{-1} and dose ≥0.15 mg kg^{-1}.

Table 2: Demographic features and pharmacokinetic parameters of newly diagnosed APL patients treated with arsenic trioxide in the first day of induction therapy

Pat. ID	Sex	Weight (kg)	Age (year)	Dose (mg)	Dose (mg kg^{-1})	t_{max} (h)	C_{max} (μg L^{-1})	AUC_{0-24} (μg h L^{-1})	$AUC_{0-\infty}$ (μg h L^{-1})	$t_{1/2}$ (h)	k_{el} (h^{-1})	Vd (L kg^{-1})	CL_{total} (L kg^{-1} h^{-1})
P1	F	67.0	37.0	10	0.15	2.00	46.4	701	3275	65	0.01	4.30	0.05
P2	M	74.0	29.0	10	0.14	2.00	37.3	577	1521	32	0.02	4.10	0.09
P3	F	56.0	32.0	10	0.18	2.00	16.1	243	993	57	0.01	14.80	0.18
P4	F	56.0	42.0	10	0.18	2.00	18.1	233	686	40	0.02	14.80	0.26
P5	F	60.0	15.0	10	0.17	1.00	52.4	837	2558	40	0.02	3.70	0.07
P6	M	65.0	33.0	10	0.15	1.00	35.2	513	1904	51	0.01	5.90	0.08
P7	F	85.0	24.0	10	0.12	2.00	68.4	1131	4123	49	0.01	2.00	0.03
P8	F	60.0	44.0	10	0.17	4.00	29.2	482	1321	31	0.02	5.70	0.13
P9	M	70.0	19.0	10	0.14	2.00	64.2	1089	3742	47	0.01	2.60	0.04
P10	F	45.0	15.0	10	0.22	2.00	63.4	927	2769	38	0.02	4.40	0.08
P11	F	65.0	48.0	10	0.15	2.00	59.3	916	2731	39	0.02	3.20	0.06
P12	M	90.0	27.0	10	0.11	3.00	24.2	379	1052	34	0.02	5.10	0.11
P13	F	115.0	17.0	10	0.09	2.00	74.5	1177	1690	25	0.03	1.90	0.05
P14	F	60.0	34.0	10	0.17	3.00	50.3	830	2629	39	0.02	3.60	0.06
P15	F	94.0	38.0	10	0.11	2.00	26.3	386	917	27	0.03	4.50	0.12
P16	M	51.0	31.0	10	0.20	3.00	41.4	640	2034	39	0.02	5.40	0.10
P17	M	74.0	22.0	10	0.14	2.00	49.4	738	1951	32	0.02	3.20	0.07
P18	M	76.0	47.0	10	0.13	3.00	18.6	319	1362	57	0.01	7.90	0.10
P19	M	74.0	55.0	10	0.14	1.00	22.5	363	1346	52	0.01	7.50	0.10
P20	M	48.0	18.0	10	0.21	2.00	74.5	1177	1949	43	0.02	6.70	0.11
Mean		69.3	31.4	10	0.15	2.15	43.6	683	2027	41	0.02	5.60	0.10
SD		17.0	11.9	0	0.03	0.70	19.5	317	958	10	0.01	3.60	0.05

t_{max}: Time to reach peak concentration; C_{max}: Peak plasma arsenic concentration; AUC_{0-24}: Area under the plasma concentration-time curve up to 24 h calculated by using the trapezoidal method; AUC_{0-8}: Area under the plasma concentration-time curve up to 8 calculated by adding AUC_{0-24} with AUC_{24-8}; $t_{1/2}$: Elimination half life calculated by using the formula $t_{1/2} = 0.693/K_{el}$; K_{el}: Elimination rate constant of arsenic calculated by using the slope of curve and the formula of k_{el} = -Slope×2.303; Vd: Volume of distribution calculated from Dose/(k_{el}×AUC_{0-8}) formula; Cl_{tot}: Total clearance calculated as dose/$AUC_{0-\infty}$ on day 1

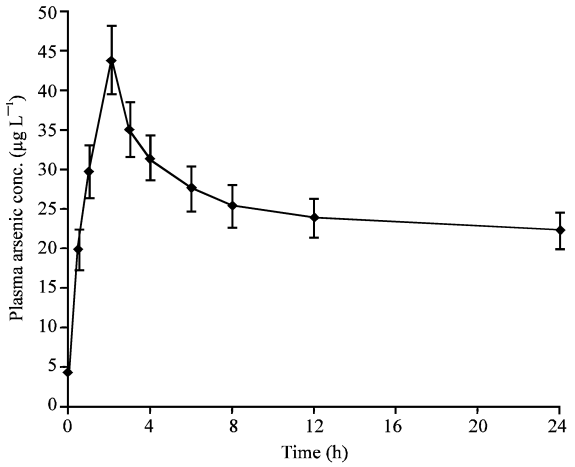

Fig. 1: Mean plasma concentration-time profile in 20 patients with Acute Promyelocytic Leukemia (APL) after a 2 h intravenous infusion of arsenic trioxide on day 1. Data are presented as Mean±SEM

The pharmacokinetic parameters of those are shown in Table 3. Statistical analysis of the parameters showed no significant differences between them. According to Table 3, considering the gender of patients showed that the plasma concentration profile of arsenic in male was similar to that in female and their pharmacokinetic parameters were not different in male (n = 9) and female (n = 11).

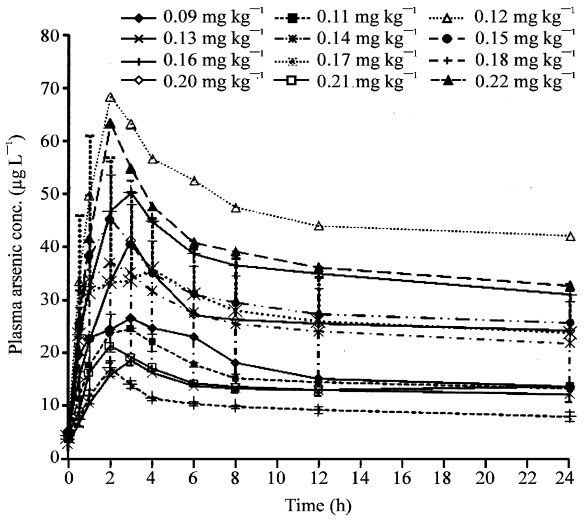

Fig. 2: Mean plasma concentration-time profile for different doses in patients with Acute Promyelocytic Leukemia (APL) after a 2 h intravenous infusion of arsenic trioxide on day 1. Data are presented as Mean±SEM

To further investigate the excretion of arsenic from the body, content of arsenic in 24 h urine samples was measured in 13 patients. The results showed that urinary arsenic content was slightly increased during drug administration and the total amount of arsenic excreted

Table 3: Mean of pharmacokinetic parameters of arsenic trioxide in twenty newly diagnosed APL patients according to sex, dosage and age of patients (n = 20)

	Sex		Dosage (mg kg^{-1})	
Parameters	Male	Female	<0.15	≥0.15
No.	9.000	11.000	9.000	11.000
Age (year)	31.000±12.5	31.000±12.0	31.000±13.0	29.000±12.0
Weight (kg)	70.000±13.0	69.000±20.4	83.000±14.0	54.000±6.0
Dose (mg kg^{-1})	0.150±0.03	0.150±0.04	0.120±0.02	0.190±0.02
C_{max} (μg L^{-1})	40.800±19.0	45.800±20.0	42.800±21.0	43.200±21.0
t_{max} (h)	2.100±0.8	2.200±0.75	2.100±0.6	2.400±0.9
AUC_{0-24} (μg h L^{-1})	644.000±310.0	714.000±334.0	684.000±360.0	682.000±295.0
$AUC_{0-\infty}$ (μg h L^{-1})	709.000±307.0	778.000±326.0	747.000±343.0	726.000±338.0
K_{el} (h^{-1})	0.370±0.25	0.510±0.3	0.440±0.3	0.440±0.3
CL_{total} (L kg^{-1} h^{-1})	0.016±0.01	0.016±0.01	0.016±0.01	0.018±0.01
Vd (L)	66.000±62.0	68.000±77.0	72.000±76.0	64.000±67.0

Data are presented as Mean±SD

Table 4: Urinary excretion of arsenic during the first day of induction therapy in 13 newly diagnosed APL patients

Patient (ID)	Urine volume (L)	Concentration (μg L^{-1})	Excretion (μg 24 h^{-1})	Excretion (%)	Cl_{renal} (mL kg^{-1} h^{-1})
P1	1.10	200.0	220	2.2	3.7
P2	1.20	135.7	156	1.6	3.4
P3	1.30	172.3	224	2.2	13.1
P4	2.10	87.6	184	1.8	12.4
P5	3.10	56.8	176	1.8	3.3
P6	2.30	85.7	197	2.0	5.4
P7	0.50	121.4	55	0.5	0.5
P8	1.70	138.8	236	2.4	7.4
P9	1.50	64.2	97	1.0	1.2
P12	1.30	95.4	124	1.2	6.5
P13	0.30	73.7	24	0.2	0.3
P16	2.10	90.3	186	1.9	4.9
P17	1.70	139.4	237	2.4	4.0
Mean	1.50	112.4	163	1.6	5.1
SD	0.80	48.1	69	0.7	4.1

daily in the urine accounted for 1.6±0.7% of the total daily dose (Table 4). The Cl_{renal} value for arsenic was 5% of Cl_{total}.

DISCUSSION

Arsenic trioxide (As_2O_3, ATO) is the oldest chemotherapeutic agent which has been used in treatment of different diseases and recently approved by FDA for the treatment of APL patients who relapsed after ATRA and/or other chemotherapy. There are promising evidences about the effectiveness of ATO as a first line therapy in newly diagnosed APL patients (Ghavamzadeh *et al*., 2006). Recent investigation shows that, among inorganic arsenic and methylated metabolites, Arsenic (III) could induce cytotoxicity against NB_4 cells that derived from APL patients, degradation of PML-RAR-α chimeric protein that formed as a consequence of the t(15: 17) and causes the pathogenesis of APL and differentiation in NB_4 cells (Chen *et al*., 2003). The high complete remission rate with arsenic trioxide (ATO) in relapsed Acute Promyelocytic Leukemia (APL) patients has been led to its use in newly diagnosed patients. In Iran, ATO has been used as a first line therapy in newly diagnosed APL patients for many years (Ghavamzadeh *et al*., 2006), but so far there has not been done any study to determine the pharmacokinetic of this drug in Iranian APL patients. So, in this study we investigated the pharmacokinetic parameters of ATO in twenty newly diagnosed APL patients. We used Graphite-furnace Atomic Absorption (GFAA) technique to analysis total arsenic in plasma and urine of patients. We set the matrix modifier and temperature program for plasma and urine samples, separately. In the present study, 17 of 20 newly diagnosed APL patients obtained CR after using As_2O_3. This result is of particular clinical significance, since previous experiences suggest that relapsed APL patients treated with ATRA and/or chemotherapy have relatively poor prognosis.

The peak concentration (C_{max}) of arsenic was achieved in plasma after 2.15 h (t_{max}) with the mean of 43.6±19.5 μg L^{-1} (range; 16.1-74.5 μg L^{-1}). This t_{max} is similar to those reported before (Shen *et al*., 2001; Wang *et al*., 2004; Fukai *et al*., 2006; Fujisawa *et al*., 2007). After t_{max}, arsenic concentration decreased slowly in plasma and retained between 20-30 μg L^{-1} in most of times. The results showed that urinary arsenic concentration in the first day of treatment was

163 μg day^{-1} (range; 24-237 μg day^{-1}) and the total amount of arsenic excreted daily in the urine accounted for approximately 0.2 to 2.4% of the total daily dose (Table 4). These results suggest that other pathways of excretion, such as through the bile, may play an important role in eliminating (removing) arsenic from the human body when challenged by high levels of As (III). The urinary excretion rate of total arsenic was low and near to which previously reported by Shen *et al*. (1997) and Fujisawa *et al*. (2007). The Cl_{renal} value for arsenic was 5% of Cl_{total}. These results indicate that renal excretion plays no significant role in the elimination of arsenic in patients receiving high dose of arsenic trioxide. Therefore, it is possible that the plasma concentrations of inorganic arsenic do not increase in patients with impaired renal function, as shown by other investigators (Remick *et al*., 2004).

There are evidences that pharmacokinetic parameters of ATO in plasma have a direct relation to its dosage. For example, (Shen *et al*., 1997, 2001) showed that compared to the results of 0.16 mg kg^{-1} ATO, C_{max} of a low dose of ATO, namely 0.08 mg kg^{-1} was nearly half of that before. In this study, the plasma against time curves of different doses of ATO had different shapes (Fig. 2) but dividing the patients to two groups of more than and less than 0.15 mg kg^{-1} showed no any significant differences between their pharmacokinetic parameters (Table 3). Finally, the present results show that the usual dose (10 mg day^{-1}) of arsenic trioxide can induce remission in more than of 85% of APL patients. However, it should be pointed out that the long-term effect of arsenic trioxide needs further investigation, despite the reports of long time survival. Therefore, we recommend the use of arsenic trioxide injection for treatment of newly diagnosed APL patients and for treatment of APL patients with relapsed from or refractory to ATRA and/or chemotherapy.

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REFERENCES

Barbui, T., G. Finazzi and A. Falanga, 1998. The impact of all-trans-retinoic acid on the coagulopathy of acute promyelocytic leukemia. Blood, 91: 3093-3102.

Campillo, N., P. Vinas, I. Lopez-Garcia and M. Hernandez-Cordoba, 2000. Determination of arsenic in biological fluids by electrothermal atomic absorption spectrometry. Analyst, 125: 313-316.

Chen, G.Q., L. Zhou, M. Styblo, F. Walton and Y. Jing *et al*., 2003. Methylated metabolites of arsenic trioxide are more potent than arsenic trioxide as apoptotic but not differentiation inducers in leukemia and lymphoma cells. Cancer Res., 63: 1853-1859.

Fujisawa, S., R. Ohno, K. Shigeno, N. Sahara and S. Nakamura *et al*., 2007. Pharmacokinetics of arsenic species in Japanese patients with relapsed or refractory acute promyelocytic leukemia treated with arsenic trioxide. Cancer Chemother. Pharmacol., 59: 485-493.

Fukai, Y., M. Hirata, M. Ueno, N. Ichikawa and H. Kobayashi *et al*., 2006. Clinical pharmacokinetic study of arsenic trioxide in an acute promyelocytic leukemia (APL) patient: Speciation of arsenic metabolites in serum and urine. Biol. Pharm. Bull., 29: 1022-1027.

Ghavamzadeh, A., K. Alimoghaddam, S.H. Ghaffari, S. Rostami and M. Jahani *et al*., 2006. Treatment of acute promyelocytic leukemia with arsenic trioxide without ATRA and/or chemotherapy. Ann. Oncol., 17: 131-134.

Grignani, F., M. Fagioli, M. Alcalay, L. Longo and P.P. Pandolfi *et al*., 1994. Acute promyelocytic leukemia: From genetics to treatment. Blood 83: 10-25.

Lengfelder, E., S. Saussele, A. Weisser, T. Buchner and R. Hehlmann, 2005. Treatment concepts of acute promyelocytic leukemia. Crit. Rev. Oncol. Hematol., 56: 261-274.

Nixon, D.E., G.V. Mussmann, S.J. Eckdahl and T.P. Moyer, 1991. Total arsenic in urine: Palladium-persulfate versus nickel as a matrix modifier for graphite furnace atomic absorption spectrophotometry. Clin. Chem., 37: 1575-1579.

Remick, S., C. Sweeney, C. Takimoto, D. Douer and A. Bernareggi, 2004. Pharmacokinetics (PK) of arsenic trioxide in cancer patients (pts) with renal dysfunction: Preliminary results. J. Clin. Oncol., 22: 2018-2018.

Shen, Z.X., G.Q. Chen, J.H. Ni, X.S. Li and S.M. Xiong *et al*., 1997. Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood, 89: 3354-3360.

Shen, Y., Z.X. Shen, H. Yan, J. Chen and X.Y. Zeng *et al*., 2001. Studies on the clinical efficacy and pharmacokinetics of low-dose arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia: a comparison with conventional dosage. Leukemia, 15: 735-741.

Tallman, M.S., C. Nabhan, J.H. Feusner and J.M. Rowe, 2002. Acute promyelocytic leukemia: Evolving therapeutic strategies. Blood, 99: 759-767.

Tallman, M.S., J.W. Andersen, C.A. Schiffer, F.R. Appelbaum and J.H. Feusner *et al*., 2002. All-trans retinoic acid in acute promyelocytic leukemia: Long-term outcome and prognostic factor analysis from the North American Intergroup protocol. Blood, 100: 4298-4302.

Wang, Z., J. Zhou, X. Lu, Z. Gong and X.C. Le, 2004. Arsenic speciation in urine from acute promyelocytic leukemia patients undergoing arsenic trioxide treatment. Chem. Res. Toxicol., 17: 95-103.

Warrell, R.P., S.R.J. Frankel, W.H.J. Miller, D.A. Scheinberg and L.M. Itri *et al*., 1991. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid). N. Engl. J. Med., 324: 1385-1393.