RESEARCH COMMUNICATION

Pharmacokinetics of Arsenic Trioxide (As_2O_3) in Chinese Primary Hepatocarcinoma Patients

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Abstract

Background: Arsenic trioxide (As_2O_3) induces growth inhibition and apoptosis in human hepatocarcinoma cell lines, but little is known about its pharmacology with this cancer in vivo. Pharmacokinetics after As_2O_3 injection into patients with a primary hepatocarcinoma (PHC) were therefore investigated. Methods: Fourteen patients were enrolled after providing informed consent and given daily intravenous doses of 10mg for 14 days. Three mL blood samples were collected before and 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 12 h and 24h after the drug infusion on days 1 and 14, as well as once every other day from day 2, for measurement of plasma concentrations using an atom fluorescent assay and analysis of pharmacokinetic parameters with the PKBP-N1 program. Results: Data from 13 cases were evaluable, 1 case being excluded due to an insufficient blood sample. Pharmacokinetics were consistent with the characteristics of the two-compartment model, parameters on days 1 and 14 being closely similar. The mean plasma maximal peak concentration (Cpmax) was 136.4±89.4μg/L, lasma distribution half-life time ($T_{1/2}\alpha$) was 0.071±0.027 hours, plasma elimination half-life time ($T_{1/2}\beta$) was 23.9±18.4 hours, apparent distribution volume (Vd) was 335.1±387.0L, entry distribution volume (Vc) was 20.3±21.3L, system clearance(CLs) was 8.65±4.26L/h, area under curve (AUC0-t) of concentration-time was 1128.5±510.3 μg·h/L. From days 2 to 14, minimal steady state plasma drug concentration (C^{ss}min) was in the range of 31.7±9.27μg/L to 55.6±32.3μg/L for 10 detected patients. Conclusions: The data suggested that a two-compartment model most accurately reflects As_20_3 pharmacokinetics in PHC patients. The apparent distribution volume was comparatively large and the plasma drug concentration was a little low, with a comparatively long drug elimination half-life, so clinical administration of the drug should be individualized for the best clinical efficacy and prevention of side effects.

Keywords: Arsenic trioxide - pharmacokinetics - primary hepatocarcinoma treatment- Chinese patients

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Introduction

Use of arsenicals in the treatment of malignant tumors has attracted much attention in recent years as studies have shown that As_2O_3 is highly effective on acute promyelocytic leukemia (APL). The response rate for APL patients is 90% with the completely remission rate being >70% (Zang et al., 2000) and safety profile favourable (Ni et al., 1997; Shen et al., 1997) . The therapeutic range has been extended to chronic myelocytic leukemia (CML), malignant lymphoma (ML), myelocytic dysfunction syndrome (MDS) and multiple myloma (MM) (Chen et al., 1996; Mervis et al., 1996). Moreover, the usefulness of As_20_3 in the treatment of many non-leukemic solid tumors has also been explored extensively.

Our interest has been in the possibility of using As_2O_3 injection in the treatment of hepatocarcinoma. A series of experiments starting from 1996 have demonstrated that As_2O_3 has selective anti-tumour activity effect on hepatocarcinoma cells (HCC) both in vitro and in vivo (Qin et al., 1998; Chen et al., 2000; Hua et al., 2004a; 2004b; 2004c). The main mechanism of As_2O_3 include inducing apoptosis of HCC, inhibiting telomerase, regulating gene expression and supressing neovascularization. In the initial trials, we have administered single agent of As_2O_3 to 28 cases with primary hepatocarcinoma (PHC), there was an objective response rate of 10.7% with improved quality of life and prolonged survival (Qian et al., 2002). These results have been repeatedly confirmed by other clinical studies in China (Hao et al., 2004; Zhang et al., 2004; Huang et al., 2005).

The liver is the major site of As_2O_3 metabolism. Most patients with PHC in China suffer poor liver conditions, the pharmacokinetic characteristics of As_2O_3 in these patients are likely affected, which different from those in the APL patients whose liver functions are relatively normal. Better understanding of the pharmacokinetics of As_20_3 in patients with liver cancer could be helpful for treatment regimens to maximize clinical efficacy and minimize toxicity and side effects. We conducted a

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pharmacokinetic study of As_2O_3 in patients of PHC with the permission of 81st Hospital Ethics Committee.

Materials and Methods

Patients

From December 2002 to May 2003, all 14 consecutive patients with PHC who were confirmed in the Oncology Center, Nanjing 81st Hospital, joined in the study voluntarily after signing an informed consent form. Patient characteristics are summarized in Table 1. One case was excluded from data analysis due to the insufficient plasma samples. Patients eligible for the trial were adults (age≥18 years) with PHC, Child-Pugh A or B, ECOG PS 0-2, quantifiable WBC≥3×10^9/L, PLT≥60×10^9/L, Hb≥8.5g/dL,TBIS≤2mg/dL, ALT and AST≤1.5 times the upper limit of normal in serum, and potential survival time≥12 weeks. Pregnant or breast-feeding women, hypertension, coronary heart disease and diabetes mellitus were excluded. Patients were also excluded if they had received prior treatment with chemotherapy drugs or arsenic drugs within 4 weeks. Individuals with allergic constitution of arsenic drugs or serious skin diseases were excluded.

Administration of As_2O_3

All patients were given a single dosage of daily doses of 10mg As_2O_3 injection (supplied by Yida Pharmaceutical Co., Ltd., Harbin, China; Lot number: 20001102) for 14 days. Each dose of As_2O_3 diluting in 500ml of sodium chloride solution was administered intravenously for 4 h.

Blood sampling

Blood samples for measurement of As_2O_3 concentration were obtained from a central line prior to administration and 1, 2, 3, 4, 5, 6, 8, 12 and 24 hours post-administration on day1 and day14. From day2 to day14, blood was collected once every other day before As_2O_3 adminstration. Blood samples (3 mL) were collected in heparinized tubes and centrifuged at 2,000 rpm for 5 min. Plasma was separated and frozen at -70°C prior to analysis.

Pre-processing of the blood sample

0.5ml blood sample was placed in a 150mL triangular flask, and then 10mL of thick perchloric acid and 4.0mL of thick nitric acid were added. The solution was heated on a electric hot plate and slated until it looked transparent and clear, then we transferred it into a 10mL test tube after it was cooled. Subsequently, the solution was placed in a container of metered volume of 10mL, then joggled and measured after 1.0mL of nitric acid and 10mL of mixed liquor of ascorbic acid (2%), including sulfourea (2%).

Instrument operation conditions

The analytical conditon of the instrument was as follows, wave length of the fluorescence (λ) was 90.23nm, electric current (A) was 40mA and the height of the atomization utensil (h) was 8cm. The lowest limit of quantitation of the instrument was 0.02ng/mL.

Establishment of standard curve

Vacant blood plasma 0.5mL was placed into a 150mL triangular flask subsequently, different volumes of 1ug/mL arsenic standard working solution were added as follows, 10μL, 20μL, 40μL,80μL, 0.2mL, 0.4mL, 0.6mL.The solutions were processed as the sample pre-processing method and were set the metered volume at 10ml to get the concentration of the samples 1μg /L, 2μg /L, 4μg /L, 8μg /L, 20μg /L, 40μg /L and 60μg /L , respectively. Then, the concentration of the samples were measured by atom fluorimetry, with 10% hydrochloric acid +0.2% ascorbic acid and sulfourea as carriers, boron potassium hydride (2%) +NaOH (0.5%) as reductant. Finally, the result was illustrated in a standard curve diagram.

Table 1. Patient Characteristics

		n	%
Gender	Male	12	85.7
	Female	2	14.3
	Age(years)	49±10	
	Course(d)	55 (2~570)	
Stage	IVA	9	64.3
	IVB	5	35.7
Hepatocirrhosis	Yes	11	78.6
	No	3	21.4
Tumor Lesions	1	5	35.7
	>1	9	64.3
Maximum Tumor Diameter (cm)	≤5	2	14.2
	>5	12	85.8
PVTT	Yes	8	57.1
	No	6	42.9
Metastasis	Yes	5	35.7
	No	9	64.3
AFP	≤20μg/L	3	21.5
	>20μg/L	11	78.5
Child-Pugh	A	9	64.3
	B	5	35.7

[a]Median (interquartile range)

Measurement of recovery rate

Vacant blood plasma 0.5ml was prepared for 5 portion to make low, middle and high blood concentration with arsenic of 4μg /L, 20μg /L and 40μg /L respectively. Then the solutions were measured with the standard curve method and their recovery rates were calculated.

Measurement of the degree of precision

Vacant blood plasma 0.5mL was prepared for 5 portion to make low, middle and high blood concentration with arsenic of 4μg /L, 20μg /L and 40μg /L respectively. Then the solutions were measured with the standard curve method and their variabilities were observed within a day and between days.

Measurement of arsenic in the blood samples

The pre-processing method was processed for the blood plasma samples of patients with PHC by atom fluorimetry, incuding of 10% hydrochloric acid+0.2% ascorbic acid and sulfocarbamide as carriers, (2%) boron potassium hydride + (0.5%) caustic soda as reductant.

Statistical Analysis

All values are presented as mean ± standard error of the mean(SEM) or median (interquartile range). SPSS 13.0

Table 2. Blood Arsenic Recovery Rates Measurement

Standard concentration (μg/L)	Actual concentration (μg/L)	Recovery rate (%)
4	3.96±0.142	99.0±3.55
20	19.45±0.352	97.2±1.625
40	40.08±0.799	100.2±1.998

for Windows was used for all of the statistical analyses performed.

Results

Methodology of determining As_2O_3 concentrations in plasma

Atom fluorimetry was chosen for the measurement of the blood arsenic concentrations. This method is believed to have the characteristics of high sensitivity, good specificity, accurate quantitation and simple operation. In our study, standard curves were routinely obtained with linear regression (Y=0.01135X +0.0578, r=0.9993) within the concentration range of 1 and 60μg/L of As_2O_3 . When blood samples containing known concentrations of As_2O_3 at low, middle and high range were used, the recovery rate of As_2O_3 from plasma was between 97.2% and 100.2% (Table 2), and the relative standard deviation was <3.59% within a day and less than 8.37% between days (Table 3).

Pharmacokinetics of intravenous doses of As_2O_3 in PHC patients

Reliable methods had been established to measure As_2O_3 in blood samples of all patients. Pharmacokinetic data was collected from 13 patients with PHC who had been detected and analyzed by PKBP-N1 program. Data of 1 patient who didn't reach the standard request was excluded because of too much deposit of fat and collagen protein in the blood plasma and shortage of plasma. Summary statistics on plasma concentrations

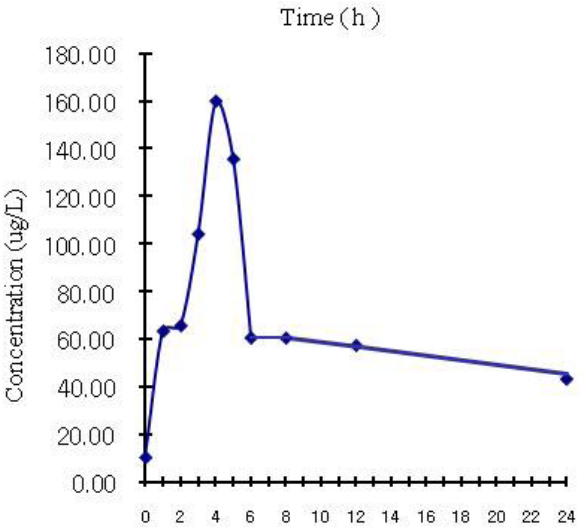

Figure 1. Time Curve Plasma for Concentrations of As_2O_3

of As_2O_3 at each of the sampling times on day 1 were presented in Table 4. and Figure 1.The data obtained on day 14 was similar to that on day 1. There were eligible data from 13 cases, which achieved the mean plasma maximal concentration (Cpmax) 136.4±89.4 μg/L, plasma distribution half-life time ($T_{1/2}\alpha$) 0.071±0.027 h ,plasma elimination half-life time ($T_{1/2}\beta$) 23.9±18.3 h, apparent distribution volume (V_d) 335.0±387.0 L, center distribution volume (Vc) 20.3±21.3 L, system clearance (CLs) 8.65±4.26 L/h and area under curve (AUC) of concentration-time 1128.5±510.2 μg·h/L.

The minimal plateau concentration (C^{ss} min)

From day 2 to da y14, the plasma As_2O_3 concentrations in 10 patients were detected immediately before administration every other day. Failure to obtain full sampling for all patients, because of no data from 4

Table 3. Investigation of the Precision Degree of the Blood Arsenic Measurement (n=5)

Standard concentration	Deviation within a day		Deviation between days	
(μg/L)	MV (μg/L)	RSD (%)	MV (μg/L)	RSD (%)
4	3.974±0.106	2.670	4.124±0.345	8.370
20	19.878±0.514	2.590	19.352±0.563	2.910
40	39.440±1.414	3.590	39.466±1.085	2.750

MV, measured value; RSD, relative standard deviation

Table 4. Pharmacokinetic Parameters for 13 Patients on Day 1

Case ID	Cp_{max} (μg/L)	$T_{1/2}\alpha$9(h)	$T_{1/2}\beta$(h)	V_d(L)	Vc(L)	CL(s)(L/h)	AUC(μg •h/L)
1	59.400	0.030	14.597	306.865	29.326	14.573	1089.900
2	49.400	0.041	25.068	445.808	19.567	12.327	778.600
3	43.400	0.036	46.843	645.538	17.375	9.554	774.300
4	282.000	0.094	12.821	86.552	6.953	4.679	1466.100
5	158.000	0.043	18.672	96.455	21.330	3.580	1227.300
6	285.000	0.107	14.252	53.068	8.592	2.581	833.600
7	198.000	0.103	10.799	137.797	6.344	8.845	1796.300
8	168.000	0.082	14.172	83.538	10.440	4.085	1799.500
9	84.200	0.082	13.568	194.911	12.280	9.958	937.200
10	174.000	0.076	12.237	136.649	8.607	7.740	388.900
11	189.000	0.103	10.105	170.494	13.732	11.694	715.700
12	55.000	0.070	55.009	540.849	87.358	6.815	207.600
13	28.000	0.059	63.030	1457.279	22.616	16.026	787.200
x±s	136.4±89.4	0.071±0.027	23.9±18.3	335.0±387.0	20.3±21.3	8.65±4.26	1128.5±510.2

Table 5. The Smallest Blood Plasma Drug Concentration and Time Data of Ten Patients (μg/L)

	Time(d)						
Case ID	2	4	6	8	10	12	14
1	31.8	35.0	35.0	44.6	52.8	51.4	48.0
2	26.0	21.0	34.2	26.6	30.0	36.4	37.0
3	35.0	38.0	35.8	37.2	29.6	39.6	30.0
6	35.6	39.4	37.8	41.2	46.6	42.2	37.2
7	52.2	85.2	56,2	121.0	78.6	70.2	54.6
9	30.1	57.3	38.4	38.4	49.2	35.0	31.6
10	16.6	39.7	23.4	36.4	38.0	30.2	19.6
11	27.0	36.8	37.4	65.4	20.2	60.0	40.0
12	36.0	50.0	101.0	86.8	126.0	142.0	54.2
13	26.8	29.4	97.2	51.0	85.0	41.2	51.0
x±s	31.71±9.27	43.18±17.81	49.64±27.27	54.86±28.93	55.60±32.27	54.82±32.98	40.32±11.56

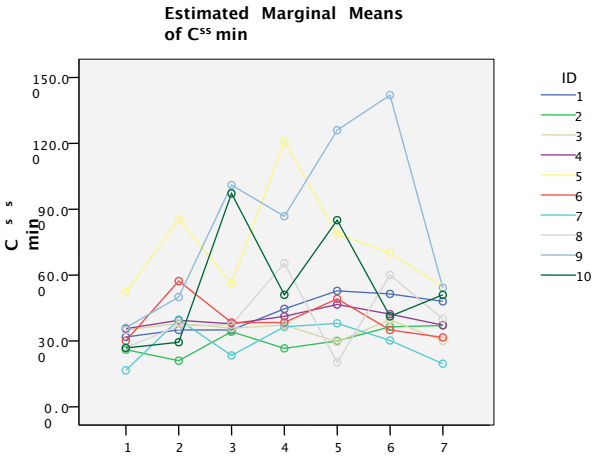

Figure 2. Smallest Blood Plasma Drug Concentration and Time Dots Data in Ten Patients

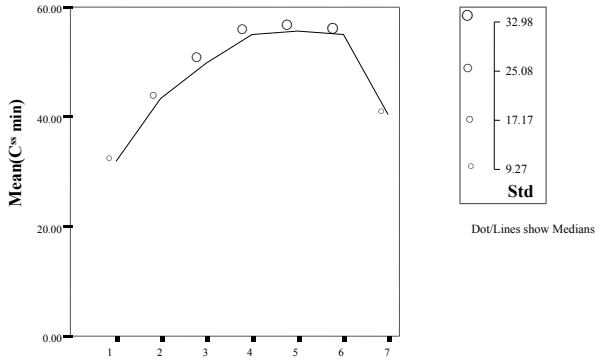

Figure 3. Smallest Blood Plasma Drug Concentrations and Time Dots Data

patients, including of the declinations (2 cases), fatty plasma (1 case) and hepatic encephalopathy (1 case). As shown in Table 5, Figure 2 and Figure 3, the average minimal plateau concentration (C^{ss}_{min}) of 10 evaluable patients was 31.71±9.27μg/L-55.60±32.27μg/L.

Efficacy of arsenic therapy

We evaluated the efficacy of arsenic trioxide by monitoring the diversity of performance status (PS), quality of life (QOL), the degree of pain remission and the degression of α-fetoprotein (AFP). Except that one failed to complete the arsenic therapy for hepatic encephalopathy, the efficacy of the others were as follows. Two patients achieved the improvement of PS and QOL. Among 6 patients with intense pain of hepatic region, 4 achieved CR and 2 PR. The AFP decreased markedly in 4 patients with metastasis. It's worth nothing that the AFP decreased to more than 50% but rose again after 3 weeks.

Adverse events

The adverse events observed in which patients undertook the pharmacokinetic study included digestive tract symptoms, hepatic and renal dysfunction, leucocytopenia and edema of lower extremity. Ten patients had hepatic and renal dysfunction of Grade I-II had digestive tract symptoms eucocytopenia of Grade I-II. However, these adverse events were not serious and could be cured with symptomatic therapy.

Discussion

In the last decade, As_2O_3 has been used to treat APL with significant efficacy and favorable safety profile. Recently, we found that As_2O_3 is also effective in the treatment of PHC that other researchers subsequently confirmed our findings. When As_2O_3 injection was administered into the body, it is metabolized and detoxicated in the liver. However, most Chinese patients with liver cancer have chronical hepatitis B and cirrhosis, which result in hepatic dysfunction to different degree. It follows that the pharmacokinetic process of As_2O_3 in patients with liver cancer is likely different from that in the APL patients whose liver functions are normal as a whole. To the best of our knowledge, this is the first report on pharmacokinetics of As_2O_3 in hepatocarcinoma patients.

In the present study, we adopted the advanced atom fluorimetry to detect plasma drug concentration. This new high-tech method was developed for trace quantity analysis, which enjoys the superiority of convenient operation, high sensitivity, good specificity and accurate quantitation over other methods. In our study, it indicates that standard curves can be rutinely constructed with good linear regression in the concentration range of 1-60μg/L As_2O_3. The recovery rate of As_2O_3 from the detected samples and the relative standard deviation between samples within a day and between days met up with the measurement requirement for biological specimen.

Our pharmacokinetic analysis indicated that intravenously administered As_2O_3 in PHC patients exhibited the characteristics which were consistent with the two-compartment model of distrbution and elimination. In PHC patients, the pharmacokinetic profile of As_2O_3 is generally similar to that in patients with APL

(Ni et al., 1997). However, liver cancer patients showed a shorter $T_{1/2}\alpha$ and doubled $T_{1/2}\beta$ when compared with those with APL. Notably, there was great individual difference in $T_{1/2}\beta$ among PHC patients with the shortest 10.1h and the longest 63h. Similarly, there are also relatively larger individual differences in apparent volume of distribution (Vd) and area under the curve (AUC) for PHC patients and no reports showed for APL patients.

Vd and AUC are closely related to plasma concentration. When Vd is larger, AUC is smaller, including the lower plasma drug concentration. All the parameters indicated that the plasma drug concentration was relatively low. This could be accounted for by the retention of interstitial fluid due to the low levels of plasma proteins and lowercolloid osmotic pressure in PHC patients. But, compared to data reported in APL patients, there are higher pharmacokinetic parameters in patients with PHC in the study.

As_2O_3 is mainly detoxicated in the liver through methylation by converting to methylarsenate and cacodylate and excreted through the kidney and intestinal tract. The present study involves PHC patients at the advanced stage with posthepatitic cirrhosis (11 cases) and abnormal liver functions. Plasma proteins are lower than normal in 4 patients. As a result, the binding capacity of plasma proteins with the drug is weakened, which leads to increased free As_2O_3 in plasma. Quick distribution and equilibration of free As_2O_3 in interstitial fluid result in shorter $T_{1/2}\alpha$. At the same time, the half-life time of the drug is prolonged because of the ability of the liver to metabolize and clear the drug. Furthermore, the clearance of the drug is also slower as the glomerular filtration slows down. For example, as a consequence of liver cirrhosis and the build-up of pressure in the portal veins, which lead to accumulation of blood in internal organs, retention of interstitial fluid, and decrease of blood volume. However, the system clearance (CLs) is not obviously lowered (8.650±4.264L/h), indicating the normal excretion function of the renal tubule in this group of patients.

It is generally thought that oral doses of 10-50mg As_2O_3 are poisonous and 60mg would be fatal. However, in our previous trial, 10-15mg As_2O_3 was administered intravenously to treat many liver cancer patients. Since no events of acute poisoning were observed, it suggests that iv administration of As_2O_3 in treating liver cancer is generally safe. The major side effects observed including slight gastrointestinal tract reaction, myelosuppression and mild liver and renal toxicities. Moreover, mild side effects were observed in lower plasma drug concentration of As_2O_3 in the study. Although As_2O_3 may not be the only contributor to injury to the liver and kidney in patients with advanced progressing disease, who should be treated with caution.

To conclusion, our pharmacokinetic study of intravenous doses of As_2O_3 in patients with PHC provides insights for guiding the clinical administration of As_2O_3 in the treatment of PHC. In the study, it indicates that high doses and repeated courses of As_2O_3 can maintain concentrations sufficiently for therapeutic efficacy, but it will result in drug accumulation and heightening clinical toxicity due to differences of half-life time, and individual differences in drug. Proper dosage of the drug may be tailored for individuals according to their ages, PS, disease stages and hepatonephric functions,which gets the best clinical efficacy and minimize side effects and toxicity. Further trials are warranted for safety and efficacy assessment.

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