RESEARCH COMMUNICATION

Pharmacokinetics of Arsenic Trioxide (As$_2$O$_3$) in Chinese Primary Hepatocarcinoma Patients

Haiqing Hua$^{1,2}$, Shukui Qin$^2$, Jianzhong Rui$^3$, Jinheng Li$^3$

Abstract

**Background:** Arsenic trioxide (As$_2$O$_3$) 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$_2$O$_3$ 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 24 h 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±89.4 μg/L, plasma 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 (Cl) 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±9.27 μg/L to 55.6±32.3 μg/L for 10 detected patients. **Conclusions:** The data suggested that a two-compartment model most accurately reflects As$_2$O$_3$ 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

Asian Pacific J Cancer Prev, 12, 61-65
pharmacokinetic study of As2O3 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≥3×109/L, PLT≥60×109/L, Hb≥8.5g/dL, TBIL≤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 As2O3
All patients were given a single dosage of daily doses of 10mg As2O3 injection (supplied by Yida Pharmaceutical Co., Ltd., Harbin, China; Lot number: 20001102) for 14 days. Each dose of As2O3 diluting in 500ml of sodium chloride solution was administered intravenously for 4 h.

Blood sampling
Blood samples for measurement of As2O3 concentration were obtained from a central line prior to administration and between days. Blood samples (3 mL) were collected in heparinized tubes and centrifuged at 2,000 rpm for 5 min. Plasma was collected once every other day before As2O3 administration. From day 2 to day 14, blood was collected once every other day before As2O3 administration. 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 condition 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 triangle 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.

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>
<thead>
<tr>
<th>Table 1. Patient Characteristics</th>
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<tbody>
<tr>
<td>Gender</td>
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<tr>
<td>Age(years)</td>
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<tr>
<td>Stage</td>
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<td></td>
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<tr>
<td>Hepatocirrhosis</td>
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<td></td>
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<tr>
<td>Tumor Lesions</td>
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<td></td>
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<td>Maximum Tumor Diameter (cm)</td>
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<tr>
<td>PVTT</td>
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<td></td>
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<tr>
<td>Metastasis</td>
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<td></td>
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<tr>
<td>AFP</td>
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<td></td>
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<tr>
<td>Child-Pugh</td>
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Median (interquartile range)
Table 2. Blood Arsenic Recovery Rates Measurement

<table>
<thead>
<tr>
<th>Standard concentration (μg/L)</th>
<th>Actual concentration (μg/L)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.96±0.142</td>
<td>99.0±3.55</td>
</tr>
<tr>
<td>20</td>
<td>19.45±0.352</td>
<td>97.2±1.625</td>
</tr>
<tr>
<td>40</td>
<td>40.08±0.799</td>
<td>100.2±1.998</td>
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Table 3. Investigation of the Precision Degree of the Blood Arsenic Measurement (n=5)

<table>
<thead>
<tr>
<th>Standard concentration (μg/L)</th>
<th>Deviation within a day</th>
<th>Deviation between days</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV (μg/L)</td>
<td>RSD (%)</td>
<td>MV (μg/L)</td>
</tr>
<tr>
<td>4</td>
<td>3.974±0.106</td>
<td>2.670</td>
</tr>
<tr>
<td>20</td>
<td>19.878±0.514</td>
<td>2.590</td>
</tr>
<tr>
<td>40</td>
<td>39.440±1.414</td>
<td>3.590</td>
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Table 4. Pharmacokinetic Parameters for 13 Patients on Day 1

<table>
<thead>
<tr>
<th>Case ID</th>
<th>C_{p_{max}} (μg/L)</th>
<th>T_{α}(h)</th>
<th>T_{β}(h)</th>
<th>V_{d}(L)</th>
<th>V_{c}(L)</th>
<th>CL(s)(L/h)</th>
<th>AUC(μg •h/L)</th>
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</thead>
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<tr>
<td>1</td>
<td>59.400</td>
<td>0.030</td>
<td>14.597</td>
<td>306.865</td>
<td>29.326</td>
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<tr>
<td>2</td>
<td>49.400</td>
<td>0.041</td>
<td>25.068</td>
<td>445.808</td>
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<tr>
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<td>0.094</td>
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<td>158.000</td>
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<td>96.455</td>
<td>5.380</td>
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<tr>
<td>6</td>
<td>285.000</td>
<td>0.107</td>
<td>14.252</td>
<td>53.068</td>
<td>8.592</td>
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<tr>
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<td>198.000</td>
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<td>3.580</td>
<td>1799.500</td>
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<tr>
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<tr>
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<td>9.554</td>
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<tr>
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<td>0.103</td>
<td>10.105</td>
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<tr>
<td>12</td>
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<td>0.070</td>
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<td>1457.279</td>
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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

Results

Methodology of determining As₂O₃ 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 As₂O₃. When blood samples containing known concentrations of As₂O₃ at low, middle and high range were used, the recovery rate of As₂O₃ 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 As₂O₃ in PHC patients

Reliable methods had been established to measure As₂O₃ 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 of As₂O₃ 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 (C_{p_{max}}) 136.4±89.4 μg/L, plasma distribution half-life time (T_{α}) 0.071±0.027 h, plasma elimination half-life time (T_{β}) 23.9±18.3 h, apparent distribution volume (V_{d}) 335.0±387.0 L, center distribution volume (V_{c}) 20.3±21.3 L, system clearance (CL) 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 day 14, the plasma As₂O₃ 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...
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 (Cssmin) 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, As2O3 has been used to treat APL
with significant efficacy and favorable safety profile.
Recently, we found that As2O3 is also effective in the
treatment of PHC that other researchers subsequently
confirmed our findings. When As2O3 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 As2O3 in
patients with liver cancer is likely different from that in the
APL patients whose liver functions are normal as a whole.

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
lineal regression in the concentration range of 1-60μg/L
As2O3. The recovery rate of As2O3 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
intravenous administered As2O3 in PHC patients
exhibited the characteristics which were consistent
with the two-compartment model of distribution and
elimination. In PHC patients, the pharmacokinetic profile
of As2O3 is generally similar to that in patients with APL
(Ni et al., 1997). However, liver cancer patients showed a shorter T1/2α and doubled T1/2β when compared with those with APL. Notably, there was great individual difference in T1/2β 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.

As2O3 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 free As2O3 in plasma. Quick distribution and normal in 4 patients. As a result, the binding capacity of abnormal liver functions. Plasma proteins are lower than advanced stage with posthepatitic cirrhosis (11 cases) and tract. The present study involves PHC patients at the cacodylate and excreted through the kidney and intestinal methylation by converting to methylarsenate and

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It is generally thought that oral doses of 10-50mg As2O3 are poisonous and 60mg would be fatal. However, in our previous trial, 10-15mg As2O3 was administered intravenously to treat many liver cancer patients. Since no events of acute poisoning were observed, it suggests that iv administration of As2O3 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 As2O3 in the study. Although As2O3 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 As2O3 in patients with PHC provides insights for guiding the clinical administration of As2O3 in the treatment of PHC. In the study, it indicates that high doses and repeated courses of As2O3 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.

References


