Determinants of cerebrospinal fluid arsenic concentration in patients with acute promyelocytic leukemia on oral arsenic trioxide therapy

Short title: CSF arsenic levels

Wing-Yan Au,1 Sidney Tam,2 Bonnie M. Fong,2 Yok-Lam Kwong.1

1: Department of Medicine, Queen Mary Hospital, Hong Kong
2: Department of Clinical Biochemistry, Queen Mary Hospital, Hong Kong

Correspondence to Y.L. Kwong, M.D., Department of Medicine, Professorial Block, Queen Mary Hospital, Pokfulam Road, Hong Kong.
Tel: (852) 2 855 4597
Fax: (852) 2 974 1165
Email: ylkwong@hkucc.hku.hk
Abstract

The extent of and factors controlling arsenic penetration into the central nervous system (CNS) remain unclear. Elemental arsenic levels in 67 paired cerebrospinal fluid (CSF) and plasma samples from 9 patients with acute promyelocytic leukemia (APL) on oral arsenic trioxide (As$_2$O$_3$), obtained during intrathecal chemotherapy (treatment of CNS APL, n=6; prophylaxis, n=3) were measured. Median arsenic levels of CSF and plasma were 95.8 (3.5–318.9) nmol/L and 498.9 (36.3–1,892.8) nmol/L. As a group, CSF and plasma arsenic was linearly correlated (P<0.001), with CSF at 17.7% the plasma level. The CSF/plasma arsenic ratio, which reflected the arsenic CSF penetration efficiency, varied significantly in individual patients (P<0.001). Repeated intrathecal chemotherapy and presence of blasts in CSF did not affect the CSF/plasma ratio. Plasma arsenic was the only significant determinant of CSF arsenic levels. CSF arsenic was present at therapeutically meaningful levels, implying that As$_2$O$_3$ therapy might be beneficial in CNS APL.

Keywords: arsenic trioxide, cerebrospinal fluid, acute promyelocytic leukemia
Introduction

Central nervous system (CNS) relapse of acute promyelocytic leukemia (APL) occurs in 1–5% of patients. The most important risk for CNS relapse is a high leucocyte count (>10x10⁹/L) at diagnosis. CNS APL fares poorly despite intrathecal/systemic chemotherapy and hematopoietic stem cell transplantation. The effective treatment/prevention of CNS APL remains unclear.

Arsenic trioxide (As₂O₃) is a standard treatment for relapsed APL. We first showed in an APL patient with CNS relapse that arsenic entered the cerebrospinal fluid (CSF) during oral-As₂O₃ treatment, a finding later confirmed independently in another patient receiving intravenous-As₂O₃. However, the factors controlling arsenic penetration into CSF are undefined.

We studied a series of APL patients receiving intrathecal chemotherapy while on oral-As₂O₃, which gave us the unique opportunity of documenting arsenic penetration into CSF, its extent and the factors controlling it.

Material and methods

Patients. Fifty consecutive APL patients with marrow relapse were referred. Initial diagnosis was made morphologically and cytogenetically and/or molecularly, and re-affirmed at relapse.

Treatment of relapse. Oral-As₂O₃ (10 mg/day) and idarubicin (6 mg/m²/day x 3) were administered until complete remission (CR), followed by idarubicin consolidation (6 mg/m²/day x 4) and maintenance (all-trans retinoic acid: 45 mg/m²/day; oral-As₂O₃: 5–10 mg/day; two weeks every two months for two years). As₂O₃ treatment was approved by the institutional review board at Queen Mary Hospital and performed in accordance with the Declaration of Helsinki.
**Post-As$_2$O$_3$ relapses.** Twenty patients developed post-As$_2$O$_3$ relapses, five of whom (cases 1, 2, 6–8, table 1) had isolated CNS relapse. Case 7 had been reported previously.\(^4\)

**Treatment of CNS relapse.** Intrathecal chemotherapy comprising methotrexate (12 mg) and cytarabine (50 mg) (2 times/week) and oral-As$_2$O$_3$ (5–10 mg/day) were administered until CSF was negative morphologically and molecularly for APL cells. Cranial irradiation was subsequently given in two patients (cases 6, 7).

**Intrathecal prophylaxis.** Owing to increased risks of CNS relapse in patients in CR3 and beyond, three patients (cases 3, 4, 9) consented to CNS prophylaxis. One CR2 patient (case 5), with a high leucocyte count (32 x $10^9$/L) at relapse, also consented to prophylaxis. Treatment comprised methotrexate (12 mg) weekly for 3 doses.

**Elemental arsenic measurement.** Elemental arsenic in paired CSF and plasma (obtained immediately after lumbar puncture) was measured by inductively-coupled plasma mass spectroscopy.\(^4,8\) Because most patients took the oral-As$_2$O$_3$ at home, the CSF and plasma sampling could not be timed with oral-As$_2$O$_3$ administration.

**Statistical analyses.** Statistical tests employed were described with individual experiments. All analyses were performed with SPSS version-14 (Chicago, IL).

**Results and discussion**

**Patients.** At a median follow-up of 14 (12–28) months, three of five patients with CNS relapse were in remission (Table 1). Three of four patients receiving intrathecal prophylaxis were in remission, after a median follow-up of 3 (2–14) months. None of the surviving patients had CNS sequelae from the leukemia or its treatment/prophylaxis.

**CSF and plasma arsenic levels.** Arsenic levels were determined in 67 paired CSF and plasma samples (median per patient: 6, 2–19). CSF arsenic ranged from 3.5–318.9 (median: 95.8) nmol/L, and plasma arsenic from 36.3–1,892.8 (median: 498.9) nmol/L. Previous
pharmacokinetic studies showed that after 10 mg of oral-As$_2$O$_3$, the peak plasma arsenic varied from 200–600 nmol/L. The plasma arsenic levels observed herein were within the expected range, with the high plasma levels reflecting peak, and low plasma levels reflecting trough, levels after oral-As$_2$O$_3$ administration.

**CSF arsenic was positively correlated with plasma arsenic.** As a group, there was a strong linear correlation between plasma and CSF arsenic (Figure 1A, P<0.001), with CSF at 17.7% the plasma level (slope of the regression line, Figure 1A). Analyses of sequential samples of individual patients showed similar correlations (Figures 1 B,C; supplemental file 1).

**Significant individual variability existed in arsenic penetration into CSF.** The CSF/plasma arsenic ratio was used as a measurement of the efficiency of arsenic penetration into CSF. Individual patients showed significant variations in their mean CSF/plasma ratios (Figure 1D).

**Repeated intrathecal chemotherapy did not affect arsenic CSF penetration.** To test whether chemotherapy damaged the meninges and increased arsenic penetration, the CSF/arsenic ratios of the first CSF sample of all patients were grouped, which provided the baseline efficiency of arsenic CSF penetration before chemotherapy. The CSF/plasma arsenic ratios of the second and subsequent CSF samples were similarly grouped. A comparison of the mean CSF/plasma ratios of sequential CSF samples showed no significant changes, implying that repeated intrathecal chemotherapy did not affect arsenic CSF penetration (Figure 1E).

**Arsenic CSF penetration was unaffected by CNS leukemia.** To investigate if meningeal leukemia might increase arsenic CSF penetration, the CSF/plasma ratios of CSF showing leukemic cells (n=38) were compared with those without leukemic cells (n=29). The results showed that meningeal leukemia did not affect CSF/plasma arsenic ratios (Figure 1F).

**Arsenic gradient in the CNS.** We documented that arsenic penetrated the CSF over a broad range of plasma arsenic levels. Previous studies showed that arsenic levels in peritoneal dialysates were identical to plasma, indicating that the peritoneum did not possess an arsenic
barrier. On the contrary, the difference between plasma and CSF arsenic implied that the blood brain barrier maintained an arsenic gradient. Expectedly, individual patients varied in the efficiency of arsenic CSF penetration. Regression analysis of the group showed that CSF arsenic was 17.7% of plasma. This fraction was not significantly altered with repeated intrathecal chemotherapy, an important reassurance that CNS arsenic toxicity would not be increased with concomitant intrathecal chemotherapy.

**Therapeutic significance of arsenic CSF penetration.** The median CSF arsenic was about 100 nmol/L, highest being >300 nmol/L. In primary APL cells, significant cytotoxicity starts with arsenic at 100–400 nmol/L, with approximately 50% cell death at 500 nmol/L.\textsuperscript{10} Because of variable CSF sampling time after oral-As\textsubscript{2}O\textsubscript{3}, some patients had low plasma and hence CSF arsenic, reflecting sampling at trough levels. However, in at least half of the CSF samples in this study, arsenic was still present at therapeutically meaningful concentrations. Given that the peak plasma arsenic after intravenous-As\textsubscript{2}O\textsubscript{3} may be several-fold that of oral-As\textsubscript{2}O\textsubscript{3},\textsuperscript{9} even higher CSF arsenic levels may be reached with better therapeutic efficacy after intravenous-As\textsubscript{2}O\textsubscript{3}, although this needs to be validated. However, the relative cardiac safety of oral-As\textsubscript{2}O\textsubscript{3} remains an important factor in considering the administration route.\textsuperscript{11} As CNS APL is unlikely to be treated with As\textsubscript{2}O\textsubscript{3} solely without intrathecal chemotherapy, the issue of whether systemic As\textsubscript{2}O\textsubscript{3} alone would be adequate for CNS disease will not be resolved.

**Implications in APL treatment.** None of the drugs at the dosages used in APL\textsuperscript{12} enters the CSF at significant levels, explaining why the CNS may be a leukemia sanctuary site. Patients at high risk of CNS relapse may therefore need intrathecal prophylaxis. Findings here suggest that prophylactic As\textsubscript{2}O\textsubscript{3} may be another option. Our routine maintenance during CR comprised oral-As\textsubscript{2}O\textsubscript{3} (5-10 mg/day) two weeks every two months for two years, which was well tolerated without untoward toxicities.\textsuperscript{13} Whether a more intensive arsenic maintenance might be beneficial for patients at high risk of CNS relapse will need to be validated.
Clinical aspects of arsenic CSF penetration. Our results showing arsenic penetration into the CSF are of obvious significance in toxicology studies of environmental/accidental arsenic poisoning. Finally, we and others have shown that aquaglyceroporin 9 is involved in transmembrane trafficking of arsenic.\textsuperscript{14,15} Its role in controlling arsenic CSF penetration awaits to be evaluated.

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Author contribution

W.Y. Au: conceived the study, treated the patients, obtained the specimens, wrote and approved the manuscript

Sidney Tam: performed the arsenic analysis, and approved the manuscript

Bonnie M. Fong: performed the arsenic analysis, and approved the manuscript

Y.L. Kwong: conceived the study, treated the patients, wrote and approved the manuscript

Conflict of interest

The University of Hong Kong holds a provisional patent on the use of oral arsenic trioxide in the treatment of leukemia. W.Y.A., S. T. and Y.L.K. are associated with or employed by the University of Hong Kong.
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Table 1. Features and treatment outcome of nine patients with acute promyelocytic leukemia receiving intrathecal chemotherapy for treatment or prophylaxis of central nervous system (CNS) leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex / Age</th>
<th>Status at treatment</th>
<th>Intrathecal chemotherapy</th>
<th>Additional treatment</th>
<th>Systemic relapse (time)</th>
<th>Outcome (follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F / 67</td>
<td>CR3 Relapse³</td>
<td>Treatment MTX + Ara-C (6)</td>
<td>None</td>
<td>Yes (12 m)</td>
<td>CR4, refused AHSCT, (14 m+)</td>
</tr>
<tr>
<td>2</td>
<td>M / 38</td>
<td>CR3 Relapse³</td>
<td>Treatment MTX + Ara-C (6)</td>
<td>None</td>
<td>Yes (13 m)</td>
<td>CR5 after AHSCT (14 m+)</td>
</tr>
<tr>
<td>3</td>
<td>M / 65</td>
<td>CR3 Normal</td>
<td>Prophylaxis MTX (3)</td>
<td>None</td>
<td>Yes (10 m)</td>
<td>Refused AHSCT, died of relapse (14 m)</td>
</tr>
<tr>
<td>4</td>
<td>M / 65</td>
<td>CR3 Normal</td>
<td>Prophylaxis MTX (3)</td>
<td>None</td>
<td>No</td>
<td>CR3 (3 m+)</td>
</tr>
<tr>
<td>5</td>
<td>M / 29</td>
<td>CR2 Normal</td>
<td>Prophylaxis MTX (3)</td>
<td>None</td>
<td>No</td>
<td>CR2 (2 m+), on As₂O₃</td>
</tr>
<tr>
<td>6</td>
<td>M / 44</td>
<td>CR2 Relapse³</td>
<td>Treatment MTX + Ara-C (19)⁴</td>
<td>Cranial irradiation</td>
<td>No</td>
<td>CR2 (24 m+)</td>
</tr>
<tr>
<td>7</td>
<td>M / 69</td>
<td>CR3 Relapse³</td>
<td>Treatment MTX + Ara-C (9)</td>
<td>Cranial irradiation</td>
<td>Yes (28 m)</td>
<td>Died of relapse (28 m)</td>
</tr>
<tr>
<td>8</td>
<td>M / 46</td>
<td>CR3 Relapse³</td>
<td>Treatment MTX + Ara-C (17)⁵</td>
<td>None</td>
<td>Yes (7 m)</td>
<td>Died of relapse (12 m)</td>
</tr>
<tr>
<td>9</td>
<td>M / 47</td>
<td>CR3 Normal</td>
<td>Prophylaxis MTX (3)</td>
<td>None</td>
<td>No</td>
<td>CR3 (3 m+)</td>
</tr>
</tbody>
</table>

M: male; F: female; CNS: central nervous system; MTX: methotrexate (12 mg); Ara-C: cytosine arabinoside (50 mg); m: months
CR: complete remission; AHSCT: autologous hematopoietic stem cell transplantation
1: Cases were arranged according to the alphabetical order of their names, not the order of presentation
2: time from first dose of intrathecal chemotherapy
3: Relapse was meningeal without demonstrable leukemic mass lesions in the CNS
4: two courses of intrathecal chemotherapy, 12 doses for first course, 7 doses for second course
5: two courses of intrathecal chemotherapy, 12 doses for first course, 5 doses for second course
Legend

Figure 1. Relationship of plasma and cerebrospinal fluid (CSF) arsenic levels in nine patients with acute promyelocytic leukemia receiving oral arsenic trioxide.

Figure 1A. Regression and correlation analysis of plasma and CSF arsenic levels, showing a linear positive correlation, with slope of the regression line at 0.177.

Figure 1B and 1C. Patients 6 and 8, showing that CSF arsenic levels paralleled the changes of the plasma arsenic levels. The plots of CSF versus plasma arsenic levels of the remaining seven patients were shown in the supplemental file.

Figure 1D. Significant variations in the CSF/plasma arsenic ratio existed in individual patients (values shown were mean ± 95% confidence interval, C.I.; one way analysis of variants, P<0.001). Analysis of the same set of data with a non-parametric test (Kruskal-Wallis test) also showed that the difference was significant (P<0.001) (data not shown).

Figure 1E. Repeated intrathecal chemotherapy did not significantly affect the CSF/plasma arsenic ratios (values shown were mean ± 95% confidence interval; one way analysis of variants, P=0.92). The confidence intervals were wide for intrathecal chemotherapies 8 – 17, because the number of patients requiring 8 – 17 intrathecal treatments was small. The range of data can also be better appreciated by plotting the mean ± standard deviation (supplemental file 2). Analysis of the same set of data with a non-parametric test (Kruskal-Wallis test) also showed that there was no significant difference (P=0.9) (data not shown).

Figure 1F. CSF/plasma arsenic ratio was unaffected by the presence of blasts in the CSF (+ve: positive; –ve: negative) (t test, P=0.94).
Figure 1

A. Correlation between plasma and CSF arsenic levels.

B. Arsenic levels in plasma and CSF over time for Case 6.

C. Arsenic levels in plasma and CSF over time for Case 8.

D. Mean ± 95% C.I. of CSF/plasma arsenic ratio for different patients.

E. Mean ± 95% C.I. of CSF/plasma arsenic ratio for patients with and without blast.

F. CSF/plasma arsenic ratio for patients with and without blast.
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