Effects of administration styles of arsenic trioxide on intracellular arsenic concentration, cell differentiation and apoptosis

We studied the effects of varying and steady-state concentrations of arsenic trioxide (As\textsubscript{O}\textsubscript{3}) on apoptosis and differentiation of several cell lines in vitro. We also studied the same effects of fluctuating vs constant concentrations of As\textsubscript{O}\textsubscript{3} in vivo in patients treated with daily 3-hour fast infusions or daily slow, continuous infusions. Intracellular concentrations of arsenic and apoptosis rate were higher whereas differentiation was reduced in cells exposed to constant concentrations of As\textsubscript{O}\textsubscript{3}.

Arsenic trioxide (As\textsubscript{O}\textsubscript{3}) is an effective treatment for acute promyelocytic leukemia (APL) and many other hematologic malignancies and solid tumors.\textsuperscript{1,2} However, a common side effect is leukocytosis which can threaten the life of the patients. The effect of As\textsubscript{O}\textsubscript{3} on inducing partial differentiation is one of the primary mechanisms of arsenic-related leukocytosis. We examined whether constant concentrations of As\textsubscript{O}\textsubscript{3} are associated in vitro and in vivo with less leukocytosis.

The in vitro studies were conducted with NB4, K562 and APL cell lines. The cells were cultured in media containing varying or steady-state concentrations of As\textsubscript{O}\textsubscript{3} (provided by YI-DA Pharmaceutical Limited Company) for 24 hours. The steady-state concentration was 2 μmol/L. The concentration of As\textsubscript{O}\textsubscript{3} in the other cultures was as follows: 5 μmol/L for 0.5h, 2 μmol/L for 3h, 1 μmol/L for 9.5h, and then washed out to 0 μmol/L for the remaining 12h. The varying concentrations of As\textsubscript{O}\textsubscript{3} were established on the basis of previous research.\textsuperscript{3}

The in vivo studies were approved by the ethics committee of Heilongjiang province. Two groups of patients were investigated. White cell counts, level of anemia, ratios of blast cells in the blood circulation, the age and sex of patients in the two groups were well matched. Once informed consent had been given, patients were randomized to enter the trial or control group. Intracellular arsenic concentrations, apoptosis rates and CD33/CD11b\textsuperscript+ ratios were assayed 24 hours after initiation of the first As\textsubscript{O}\textsubscript{3} infusion (just prior to the second As\textsubscript{O}\textsubscript{3} infusion).

The trial group was formed of 37 patients (acute promyelocytic leukemia, [APL] n=20, acute myeloid leukemia, [AML-M\textsubscript{4}] n=5, chronic myeloid leukemia, [CML] n=12) who were treated with a continuous slow intravenous infusion of As\textsubscript{O}\textsubscript{3}. The daily dose of As\textsubscript{O}\textsubscript{3} was 0.16 mg/kg of body weight diluted in 5% glucose 500 mL. The infusion speed was 8 drops/min, such that the whole infusion took about 18-21 hours to deliver.

The control group was formed of 38 patients (APL: 20, AML-M\textsubscript{4}: 6, CML:12) who received the same total dosage of As\textsubscript{O}\textsubscript{3} at the same dilution as that in the trial group, but administered as 45-55 drops/min, such that the whole infusion was completed in about 2-3 hours daily.

The apoptosis rates and CD33/CD11b\textsuperscript+ ratios were assayed by flow cytometry (Becton Dickinson). The intracellular arsenic concentration was measured by...
were higher than those of cells exposed to varying
inhibited differentiation and promoted apoptosis. Cells in
in vitro infusion. In consequence, this regimen should give
at vary-
constant As
infusion regimen. Table 1 shows the
varied As
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ratios can be used to evaluate differenti-
peak level (5-7
showed
to
infusions, causing a
varied As
constant As
ratios in trial patients and control patients. 

Note: the trial group compared with the control group. *p 0.01, **p< p0.05.

Table 1. Intracellular arsenic concentrations, apoptosis rates and CD33/CD11b ratios (mean±SD) n=1x10^6.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Concentrations of intracellular As (µg/L)</th>
<th>Percentage of apoptosis (%)</th>
<th>CD33/CD11b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed to constant AsO_3 conc.</td>
<td>Exposed to varied AsO_3 conc.</td>
<td>t-value</td>
</tr>
<tr>
<td>NB4</td>
<td>3.4±0.88</td>
<td>17.6±0.88</td>
<td>5.51*</td>
</tr>
<tr>
<td>K562</td>
<td>18.6±1.12</td>
<td>9.2±0.64</td>
<td>6.27*</td>
</tr>
<tr>
<td>APL</td>
<td>28.8±0.64</td>
<td>15.2±1.04</td>
<td>3.65**</td>
</tr>
</tbody>
</table>

Note: cells in changing media compared with those in 2 µM/L constant media *p<0.01, **p<0.05.

Table 2. The intracellular arsenic concentrations, the apoptosis rates and the CD33/CD11b ratios in trial patients and control patients.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Intracellular As (µg/L)</th>
<th>t-value</th>
<th>Percentage of apoptosis (%)</th>
<th>CD33/CD11b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial</td>
<td>Control</td>
<td>Trial</td>
<td>Control</td>
</tr>
<tr>
<td>APL</td>
<td>26.6±2.5</td>
<td>12.3±2.1</td>
<td>5.38*</td>
<td>28.5</td>
</tr>
<tr>
<td>AML-M</td>
<td>15.5±3.1</td>
<td>5.5±2.3</td>
<td>6.27*</td>
<td>9.5</td>
</tr>
<tr>
<td>CML</td>
<td>18.5±2.3</td>
<td>8.5±2.7</td>
<td>5.86*</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Note: the trial group compared with the control group. *p 0.01, **p< p0.05.

atomic fluorescence assay (AFS-820, China).

Data were recorded as mean ± standard deviation; the
t-test and X^2-test were used. p-values less than 0.05 were
considered statistically significant.

In vitro a positive correlation was found between intra-
cellular arsenic concentration and apoptosis rate (correla-
tion coefficient r=0.761) (Table 1). The intracellular arsenic
concentrations and apoptosis rates of leukemia
cells incubated in a constant concentration of 2 µmol/L
AsO_3 were higher than those of cells exposed to varying
concentrations of AsO_3, despite the initial level of AsO_3
being 5 µmol/L in the latter system. The ratio of
CD33/CD11b cells was higher following AsO_3 at vary-
ing concentrations than after exposure to a constant
concentration of 2.0 µmol/L AsO_3, suggesting that cells incu-
bated in varying AsO_3 concentrations had a high tenden-
cy to differentiate.

In vivo, samples were isolated from peripheral blood of
newly diagnosed leukemia patients at baseline before
received AsO_3 treatment and again 24 hours after initia-
tion of the first AsO_3 infusion (just prior to the second
AsO_3 administration).

The intracellular arsenic concentrations and the apo-
pitosis rates in the trial group were higher than those in the
control group. The proportion of CD33/CD11b cells in
the trial group was lower than that in the control group
(Table 2).

The effects of AsO_3 on leukemia cells of inducing par-
tial differentiation at low concentrations (0.1–0.5 µmol/L)
and promoting apoptosis at high concentrations, and the
pharmacokinetics of usual rate AsO_3 infusions, causing a
high AsO_3 peak level (5-7 µmol/L) and fluctuating but
diminishing concentrations in blood circulation, means
that the arsenic concentration that effectively promotes
apoptosis is maintained for less than 12h after each AsO_3
infusion, thus giving leukemia cells the opportunity to
recovery and survive. On this basis we investigated the
effects of varying concentrations and the steady-state
concentration of AsO_3 in vitro and in vivo by using a con-
tinuous slow AsO_3-infusion regimen. Table 1 shows the
relationship between constant AsO_3 exposure and
increased rate of apoptosis. Since CD33 is the surface
marker of primitive cells and CD11b is the marker of
bone marrow leukocytes, changes of CD33/CD11b
ratios can be used to evaluate differentiation. The con-
tinuous slow intravenous infusion of AsO_3-inhibited differentiation and promoted apoptosis. Administered
continuously in this manner, AsO_3 showed a curve, with a marked peak, and apoptosis-promoting
concentrations were maintained in the blood circula-
tion for long periods by the continuous micro-dosage
AsO_3-infusion. In consequence, this regimen should give
maximal therapeutic benefit and diminish leukocytosis,
confirming previous clinical results.

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Key words: arsenic trioxide, steady-state, leukocytosis, apoptosis.

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References


Acute Lymphoblastic Leukemia

Expression of the glucocorticoid receptor and its isoforms in relation to glucocorticoid resistance in childhood acute lymphocytic leukemia

In vitro prednisolone resistance is a poor prognostic factor in the treatment of childhood acute lymphoblastic leukemia (ALL). In a cohort of 54 children with ALL, a lower expression of the glucocorticoid receptor (GR), but not the relative expression levels of the GR-α, GR-β and GR-P isoforms, was associated with in vitro prednisolone resistance.

In vitro and in vivo glucocorticoid (GC) resistance are poor prognostic factors in the treatment of childhood acute lymphoblastic leukemia (ALL). In vitro resistance is an adverse risk factor, even for patients with a good in vivo response. Glucocorticoid-induced apoptosis is initiated after GC binds to the glucocorticoid receptor (GR), and hence a low level of expression of the GR has been hypothesized to be associated with GC resistance. Dexamethasone-binding studies did indeed show that a low receptor number correlated with a high rate of induction failure and relapse in childhood ALL. In contrast, two recent studies suggested that the level of GR expression is not linked to in vitro and in vivo GC resistance in childhood ALL.

Besides the functional GR-α isoform, the GR gene also encodes for isoforms that are unable to bind GC, i.e. GR-β and GR-P (Figure 1). Some studies reported a dominant negative effect (GR-β) and positive effect (GR-P) on GR-α function, whereas other studies could not confirm these findings. In the present study we investigated the correlation between the absolute expression level of GR as well as its isoforms and cellular resistance to GC. To this aim, GR-α, GR-β and GR-P mRNA levels were measured in 54 primary pediatric ALL samples using a quantitative real-time reverse transcription polymerase chain reaction strategy as previously described. Forty-two of these patients were eligible for a paired analysis of prednisolone-sensitive versus prednisolone-resistant cases matched for age (1-9 years and >10 years), immunophenotype (T- or precursor-B ALL) and white blood cell count at diagnosis (<50×10^9/L and >50×10^9/L). Patients with t(9,22) and t(4,11) positive ALL were excluded. Patients were defined as being in vitro prednisolone-sensitive or resistant by the MTT assay, using the same criteria for sensitivity (LC50 <0.1 μg/mL) and resistance (LC50 >150 μg/mL) as previously observed to be of prognostic value.

Our data revealed that GR-α is the predominant isoform, representing 71% of total GR expression. The mRNA level of GR-α strongly correlated with the protein level determined by Western blotting using a polyclonal anti-human GR antibody and anti-β-actin to control for equal protein loading (Spearman’s correlation 0.950, p<0.01, n=9). The expression of GR-β and GR-P isoforms accounts for 0.1% and 29% of total GR expression, respectively, which is comparable to levels reported in the literature. Since the expression of GR-β is very low,