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_2O_3) on apoptosis and differentiation of several cell lines *in vitro*. We also studied the same effects of fluctuating vs constant concentrations of As_2O_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_2O_3.

haematologica 2005; 90:1277-1279
(*http://www.haematologica.org/journal/2005/9/1277.html*)

Arsenic trioxide (As_2O_3) is an effective treatment for acute promyelocytic leukemia (APL) and many other hematologic malignancies and solid tumors.[1-3] However, a common side effect is leukocytosis which can threaten the life of the patients. The effect of As_2O_3 on inducing partial differentiation is one of the primary mechanisms of arsenic-related leukocytosis. We examined whether constant concentrations of As_2O_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_2O_3 (provided by YI-DA Pharmaceutical Limited Company) for 24 hours. The steady-state concentration was 2 μmol/L. The concentration of As_2O_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_2O_3 were established on the basis of previous research.[4]

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 $CD33^-/CD11b^+$ ratios were assayed 24 hours after initiation of the first As_2O_3 infusion (just prior to the second As_2O_3 infusion).

The trial group was formed of 37 patients (acute promyelocytic leukemia, [APL] n=20, acute myeloid leukemia, [AML-M_2] n=5, chronic myeloid leukemia, [CML] n=12) who were treated with a continuous slow intravenous infusion of As_2O_3. The daily dose of As_2O_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_2: 6, CML:12) who received the same total dosage of As_2O_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 $CD33^-/CD11b^+$ ratios were assayed by flow cytometry (Becton Dickinson). The intracellular arsenic concentration was measured by

Table 1. Intracellular arsenic concentrations, apoptosis rates and $CD33^-/CD11b^+$ ratios (mean±SD) n=1x10⁶.

	Concentrations of intracellular As (μg/L)			Percentage of apoptosis (%)			$CD33^-/CD11b^+$		
Cell type	Exposed to constant As_2O_3 conc.	Exposed to varied As_2O_3 conc.	t-value	Exposed to constant As_2O_3 conc.	Exposed to varied As_2O_3 conc.	X^2-value	Control	Exposed to constant As_2O_3 conc.	Exposed to varied As_2O_3 conc.
NB4	3.4±0.88	17.6 ±0.88	5.51*	56.6	23.2	49.8*	5.5±0.4	19.5±0.5	51.5±0.4**
K562	18.6±1.12	9.2±0.64	9.69*	27.6	11	21.3*	7.1±0.5	14.5±0.6	37.5±0.8**
APL	28.8±0.64	15.2±1.04	3.65**	52.2	21	43.9*	4.2±0.3	18.5±0.6	46.5±0.5**

*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.

	Intracellular cell As (μg/L)		t-value	Percentage apoptosis (%)		X^2-value	$CD33^-/CD11b^+$		
Diagnosis	Trial	Control		Trial	Control		Self-blank	Trial	Control
APL	26.6±2.5	12.3±2.1	5.38*	28.5	8.5	13.2*	5±0.5	29.5±0.5**	53.55±0.8
AML-M_2	15.5±3.1	5.5±2.3	6.27*	9.5	2.9	3.9**	3.2±0.2	28.5±0.6**	48.5±0.9
CML	18.5±2.3	8.5±2.7	5.86*	12.5	4.5	4.2**	7.5±0.5	23.5±0.5**	67.5±0.8

*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 intracellular arsenic concentration and apoptosis rate (correlation 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 As_2O_3 were higher than those of cells exposed to varying concentrations of As_2O_3, despite the initial level of As_2O_3 being 5 μmol/L in the latter system. The ratio of $CD33^-/CD11b^+$ cells was higher following As_2O_3 at varying concentrations than after exposure to a constant concentration of 2.0 μmol/L As_2O_3, suggesting that cells incubated in varying A_2O_3 concentrations had a high tendency to differentiate.

In vivo, samples were isolated from peripheral blood of newly diagnosed leukenia patients at baseline before received As_2O_3 treatment and again 24 hours after initiation of the first As_2O_3 infusion (just prior to the second As_2O_3 administration).

The intracellular arsenic concentrations and the apoptosis 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 A_2O_3 on leukemia cells of inducing partial differentiation at low concentrations (0.1–0.5 μmol/L) and promoting apoptosis at high concentrations,[4] and the pharmacokinetics of usual rate As_2O_3 infusions, causing a high As_2O_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 As_2O_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 As_2O_3 *in vitro* and *in vivo* by using a continuous slow As_2O_3 infusion regimen. Table 1 shows the relationship between constant As_2O_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 $CD33^+/CD11b^-$ to $CD33^-/CD11b^+$ ratios can be used to evaluate differentiation.[5] The continuous slow intravenous infusion of As_2O_3 inhibited differentiation and promoted apoptosis. Administered continuously in this manner, As_2O_3 showed a curve, without a marked peak, and apoptosis-promoting concentrations were maintained in the blood circulation for long periods by the continuous micro-dosage As_2O_3 infusion. In consequence, this regimen should give maximal therapeutic benefit and diminish leukocytosis, confirming previous clinical results.[6]

Jin Zhou, Ran Meng,° Xinhua Sui,#*
*Lu Meng,@ Jianping Jia,° Baofeng Yang**

*First Hospital of Harbin Medical University, China;**
Xuanwu Hospital of Capital Medical University, China;°
Mu Danjiang Medical College;# Biochemistry & Molecular Biology Department, University of Georgia, USA@

Funding: this research received financial assistance from the National Natural Science Foundation (approval number: 30370507) and Hei longjiang Excellent Youth Foundation (approval number: 2003AA9CS188-12).

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

Correspondence: Jin Zhou, M.D., Ph.D., 150001, The First Hospital of Harbin Medical University, Harbin, China. E-mail: jinzhouh85@163.com

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