Arsenic trioxide entered cerebrospinal fluid with the help of mannitol overwhelm the meningeal relapse of acute promyelocytic leukemia

Haematologica 2007; 92:(9)e82-e84 DOI: 10.3324/haematol.11826

Central nervous system (CNS) relapse of acute promyeloytic leukemia (APL) is increasingly reported after treatment with arsenic trioxide (As_2O_3),[1] the optimal therapy for this case remains unclear. As_2O_3 highly effective on APL marrow relapse, but its efficacy in CNS relapse is undefined. Recent researches showed that little As_2O_3 passed through the blood-brain-barrier (BBB) with general intravenous infusion.[2,3] which limited its use in CNS relapse of APL (CNSAPL). After discovered the different tolarance between APL blasts and human cortex neuron to As_2O_3 *in vitro*,[4,5] and primarily cleared the safe range of As_2O_3 concentration in CNS,[5-7] we created a non-invasive method to help As_2O_3 enter into CNS,[8] which indicated that 20% mannitol intravenous bolus at the speed of 0.2±0.5 mL/s opened BBB temporarily . Can this method increase arsenic concentration to therapeutic and safe level in CNS? Can As_2O_3 be used to prevent and treat CNSAPL? The treatment of a patient with isolated meningle APL relapse and chemotherapy resistance gave us the unique opportunity of documenting CNS penetration of As_2O_3 helped by mannitol intravenous bolus, which included 125 ml of 20% mannitol bolus through medial cubital vein with the speed of 12±30 mL/min, and followed with 250 mL mixed liquor (including 20% mannitol and As_2O_3 0.08 mg/kg/d) intravenous infusion with the speed of 6 mL/min, subsequently followed by As_2O_3 0.08 mg/kg/d + 5% glucose 250 mL infusion with the speed of 0.5 mL/min, the total dosage of As_2O_3 is also 0.16 mg/kg/d.

A 34-year-old woman developed APL eight years ago, experienced two times of marrow relapse and one time of CNS relapse, and achieved complete remission after treated with As_2O_3 (0.16 mg/kg/d) general intravenous infusion and ATRA oral, supplemented by mitoxantrone for leukocytosis and intrathecal methotrexate (12 mg/dose) and cytarabine (50 mg/dose). The CSF was gradually normalized (CR3).Three months after CR3, the patient represent a heavy headache, the number of blasts in CSF was 1500/L, and the intracranial pressure was 2.8 kPa. Head magnetic resonance imaging (MRI) scan showed no local lesion. Marrow examination was normal. After accepted six-days As_2O_3 general intravenous and two times of intrathecal methotrexate (12 mg/dose) and cytarabine (50 mg/dose), her symptoms and signs were not improved, and the intracranial pressure reached to 3.6 kPa, the number of APL blasts in CSF increased to 2050/L. It was clear that chemotherapy resistance occurred. We gave her the mannitol assisted As_2O_3 penetration therapy after obtained an informed consent. Spinal fluid was collected by lumber pouncture at the 30 min after As_2O_3 infusion finished every three-day. Arsenic levels in CSF were monitored dynamically by atomic fluorescence method. The apoptosis rates and $CD33^-/CD11b^+$ ratios of APL blasts were assayed by flow cytometry. The morphologic and agarose gel electrophoresis were used to evaluated apoptosis.

Results

1. Dynamic changes of arsenic levels in CSF was showed in Figure 1. Figure 1 revealed dynamic changes of arsenic in CSF with two different As_2O_3 regimen treatment, which indicated that there was a significantly arsenic leukemia-infiltrated BBB penetration with the help of mannitol than general intravenous infusion, and the arsenic in CSF reached to the inducing APL cell differentiation needed level.[9,10]

2. The morphological changes of blast cells in CSF were showed in Figure 2, which indicated that blast cells in CSF gradually went to differentiation and finally apoptosis after mannitol assisted As_2O_3 penetrating treatment,and in CSF collected in the last two times, no blast cell had been seen. The patient achieved a complete remission again (CR4).

3. The apoptosis rate and $CD33^-$/ $CD11b^+$ ratios of APL blasts were recorded in Table 1.

Since CD33 is the surface marker of primitive cells and CD11b is the marker of bone marrow leukocytes,[11] changes of $CD33^+/CD11b^-$ to $CD33^-/CD11b^+$ ratios can be used to evaluate differentiation, and $CD33^-/CD11b^-$ might indicate cellular apoptosis. Table 1 and Figures 3 and 4 revealed that intrathecal chemotherapy during the third relapse mainly promoted apoptosis, while mannitol assisted As_2O_3 penetration during the fourth relapse mainly induced differentiation.

4. The DNA ladder of blasts in CSF were showed in Figure 4. From Figure 4 (A,B) we can noticed that mannitol assisted As_2O_3 penetration mainly induced CNS APL cells partial differentiation and finally apoptosis. Intrathecal chemotherapy promote the CNS APL cells apoptosis remarkably.

5. Up to now, 18 months after CR4 followed up, the patient remained in remission, no insidious deterioration of memory and cognitive function, MMSE score was 28. MRI scan indicated no leukoencephalopathy lesion and

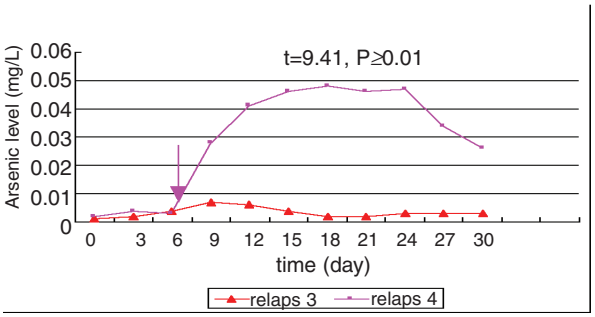

Figure 1. As_2O_3 concentration in CSF.
Note: General speed As_2O_3 intravenous infusion vs Mannitol assisted As_2O_3 penetration.

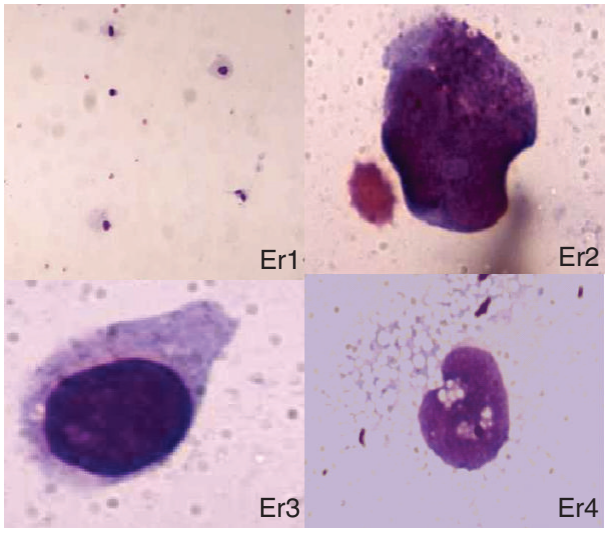

Figure 2. Morphology changes of blast cells in CSF before and after mannitol assisted As_2O_3 penetration during the forth relaps . Centrifuge smear, HE stain. Er1: baseline 1_50; Er2; baseline 1_200; Er3: the 3th time collected CSF by lumber puncture after mannitol assisted As_2O_3 penetrating treatment for 9 days; Er4: the 6th time collected CSF by lumber puncture after mannitolassisted As_2O_3 penetrating treatment for 18 days.

local neurologic lesion. No detectable PML-RARA was in peripheral blood and in the CSF.

Discussions

Since BBB prevents the entrance of water-solubility metals into the CSF,[13] the inability of most chemotherapeutic agents and As_2O_3 to adequately penetrate the BBB in either normal or tumor-infiltrated brain, is a major factor limiting the use of As_2O_3 in CNSL. Reported researches indicated that As_2O_3 general speed intravenous infusion could not make the arsenic in CSF to the therapeutic level.[4,11] In our case, although maintance As_2O_3 prevented marrow recurrence, CNS relapse still occurred and the level of element arsenic in CSF was very low. It was clear that As_2O_3 general intravenous infusion could not efficiently enter into CNS and prevent CNSL. Although meningeal leukemia had anatomical/functional breach of the BBB, the As_2O_3 penetrating ability was limited and could not reach to the effectively therapeutic level (showed in Figure 1, the line of relaps3).

This barrier, however, can be opened in a reversible manner by the intra-arterial administration of hyperosmotic agents such as mannitol,[14] but the performance of intra-arterial therapeutic related internal carotid artery cannulation and accelerated injection was beyond the tolerances of many leukemic patients who usually had severe bleeding tendency and blood coagulative disfunction.

We used 20% mannitol_Mr182, 1.0-1.5 g/kg , temperature 35-37°C peripheral intravenous injection at a speed of 12-30 mL/min opened the BBB of rabbit previously.[8] In this case, we further verified that As_2O_3 could enter into the CSF and reach to significantly therapeutic level by mannitol peripheral intravenous injection assisting.

Figure 1 and 2 revealed that although both the total

Table1. The apoptosis rate and $CD33^-/CD11b^+$ ratios of APL blasts in CSF.

Lumber puncture	Apoptosis percentage		X^2-value	$CD33^-/CD11b^+$		X2-value
	Relapse 3	Relapse 4		Relapse 3	Relapse 4	
Baseline	2.1	2.5	0.01	1.2	1.5	0.00
After treated	chemotherapy	As_2O_3				
1	5.7	4.8	0.03	1.6	1.5	0.00
2	10.5	5.5*	4.14	2.7	13.5*	6.33
3	37.6	9.4**	17.88	5.5	31.6*	9.87
4	58.5	17.5**	15.32	3.5	42.3**	18.64
5	69.5	44.6*	7.53	4.2	54.1*	17.95
6	82.2	64.7*	5.12	2.3	26.1*	9.11

*Note: relapse 3 vs relapse 4, *P≥0.05, **P≥0.01*

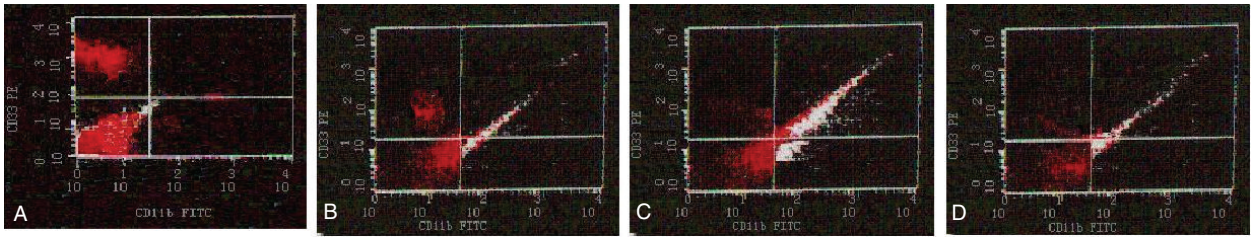

Figure 3. Dynamic changes of cellular membrane immunophenotype during mannitol assisted As_2O_3 penetration.
Note: Figure 3-A is baseline, Figure 3-B to D are the 3, 6, 9 days after accepted mannitol assisted As_2O_3 penetration.

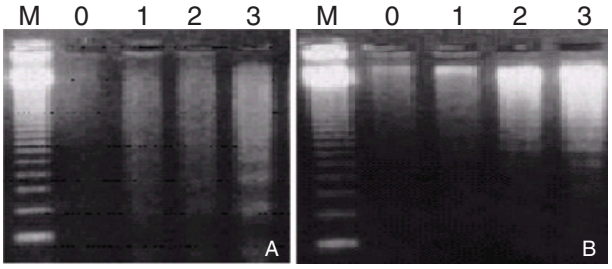

Figure 4. DNA ladders of blast cells in CSF. Note: Figure 4-A is the DNA ladders of blasts treated with intrathecal chemotherapy during the third relapse. M is the marker, lane 0 is the baseline, lane 1_3 represent to the results of the first,second and third time CSF collection by lumbar puncture after intrathecal chemotherapy. Figure 4B is the DNA ladders of APL blasts treated with mannitol assisted As_2O_3 penetration. M is the marker, lane 0 is the baseline, lane 1_3 were represent to the first, second and third time CSF collection after 3, 6 and 9 days of mannitol assisted As_2O_3 penetration.

As_2O_3 dosage daily of the two regimen were the same, the elemental arsenic levels in CSF were different. Comparing with the general As_2O_3 intravenous infusion, the *mannitol assisted As_2O_3 penetration* followed by the *slow-speed continuous As_2O_3 intravenous infusion* can not only increase the elemental arsenic concentration in CNS, but also keep the plasma arsenic to the prolonged effectively therapeutic level, and without remarkable plasma arsenic peak, which was more benefit to CNSAPL, as well as increases the prevention and treatment efficiency of APL marrow relapse, and less side-effects to normal tissues.

As the prognosis of relapsed APL remains very good, the appropriate use of treatment with minimal toxicity commensurate with long-term survival is an important priority. Our observations have important therapeutic implications for chemotherapy resistant CNS APL.

Because of the initial uncertainly whether arsenic could enter the CNS, we treated the patient conventionally with intensive intrathecal chemotherapy during the first six-days during the forth relaps. In hindsight, the combined use of chemotherapy and mannitol assisted As_2O_3 penetration did not lead to the progressive congnitive deterioration. Given that arsenic penetrated the CSF very well, it might have been possible for us to use a less intensive regimen of intrathecal chemotherapy and mannitol assisted As_2O_3 penetration to control CNSL. However, the number of volunteers was limited, further works should be down in the future. All in all, our results suggested that the mannitol assisted As_2O_3 penetration can increase the arsenic concentration in CNS to an effectively therapeutic level to CNSAPL.

Z. Jin,[1] M. Ran,[1,2] Z. Man[2]

[1]The First Hospital of Harbin Medical University, Harbin 150001, China; [2]Shijitan Hospital, The Ninth Clinical Medical College of Peking University, Beijing, China

Correspondence: Meng Ran (victor-meng@126.com)

References

1. Au WY, Kumana CR, Kou M, et al. Oral arsenic trioxide in the treatment of relapsed acute promyelocytic leukemia. Blood. 2003,102:407-8.
2. Zhou Jin, Mrng Ran, Wang Yan, et al. Clinical observation and following up of two administration methods of arsenic trioxide in treatment of acute promyelocytic leukemia. Natl Med J China, 2004, 84: 405-8
3. Zhang Peng; Wang Shuye; Hu Longhu et al. Seven years' summary report on the treatment of acute promyelocytic leukemia with arsenic trioxide-An analysis of 242 cases. Chinese Journal Of Hematology 2000; 2:10-3
4. Zhou Jin, Mrng Ran, Guan Xiuru, et al. Relationship between intracellular arsenic concentration and arsenic trioxide therapeutic sensitivity. Chin Pharm J 2004; 39:193-5
5. Zhou Jin, Meng Ran, Sui Xinhua, et al. Various tolerances to arsenic trioxide between human cortical neurons and leukemic cells. Science in China Series C 2006; 49:567-72.
6. Hou Jin, Meng Ran, Song Xueming, et al. Relationship between intracellular concentration and sensitivity to arsenic trioxide in malignant tumors and neurons. Natl Med J China 2004; 84:23-5
7. Zhou Jin, Mrng Ran, Li Limin, et al. Protective Effect of Ganglioside on Human Cortical Neuron Treated by Arsenic Trioxide. Chin Pharm J 2006; 41:27-32
8. Meng R, Zhou J, Wang D S, et al. Study on mannitol osmotic opening blood brain barrier by peripheral intravenous infusion. J Apoplexy and Nervous Disease 2003; 20: 350-2
9. Chen Z, Wang Z Y, Chen S J. Acute promyelocytic leukemia:cellular and molecular basis of differentiation and apoptosis. Pharmacol Ther 1997; 76:141-9
10. Zhou J, Meng R, Sui Xinhua, et al. Effects of administration styles of arsenic trioxide on intracellular arsenic concentration, cell differentiation and apoptosis. Haematologica 2005; 90:1303-5
11. Zhou J, Meng R, Wang Y, et al. Clinical observation and following up of two administration methods of arsenic trioxide in treatment of acute promyelocytic leukemia. Natl Med J China 2004; 84: 9-12
12. Wu MH, Smith SL, Dolan ME. High efficiency electroporation of human umbilical cord blood CD34+ hematopoietic precursor cells. Stem Cells 2001; 19:492-9
13. Kozler P. Osmotic opening of the hematoencephalic barrier in experiments. Review Rozhl Chir 2001; 80:393-6.
14. Kozler P, Pokorny J. Evans blue distribution in the rate brain after intracarotid injection with the blood-brain barrier intact and open to osmosis. Sb Lek 2003; 104:255-62