

New Treatment for Acute Promyelocytic Leukemia

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Introduction of all-trans retinoic acid (ATRA) into the treatment of acute promyelocytic leukemia (APL) provides a successful model of differentiation therapy in malignancies. Improvement and rational combination of ATRA with chemotherapy for remission induction and post-remission treatment has resulted in a complete remission rate as high as 89%⁽¹⁾ to 95%⁽²⁾ and a longer remission compared to those obtained with conventional chemotherapy. The problem that remains and interferes in longer remission is primarily early tolerance to the ATRA treatment and relapse resulting from ATRA-induced remission, usually resistant to further treatment, even with intensive chemotherapy. 9-cis retinoic acid was thought to be a potential agent that could reverse acquired resistance to retinoid because it possesses high affinity for RA receptors, particularly retinoid receptor X, but the result in clinical trial was disappointing. Tobita et al⁽³⁾ introduced a new synthetic retinoid designated Am80 in the treatment of APL in relapse. It is an agent 10 times more potent than ATRA as an in vitro differentiation inducer, it has low affinity for cellular retinoic acid binding protein, and it does not bind to retinoic acid receptor gamma. Of the 24 patients treated with this agent, 14 (54%) achieved CR. Therefore, it will be a promising compound in the treatment of relapsed APL. But the most important new finding in the treatment of newly diagnosed and relapsed APL is the introduction of arsenic compounds.

1. Historical review

At the middle of 19th century, arsenic compound had already been used for the treatment of chronic myelogenous leukemia in the form of Fowler's solution (potassium arsenite). It was abandoned after the discovery of busulfan and toxicity of arsenic after long-term administration. Two arsenic compounds are used in Chinese traditional medicine. One is "Pishaung" (or white arsenic) containing essentially arsenic trioxide (As₂O₃) administered for the treatment of some skin diseases, repair of wounds in surgical conditions, and asthma. The another is "Xiong-huang" (or Realgar compound) containing essentially arsenic sulfide, usually administered in combination with other Chinese medicines (bezoar, baphicacanthus cusia) consisting of a compound named "Bezoar antitoxic pill" for the treatment of sore throat and oral and other infections. In the early 1970s, a group of clinicians at Harbin Medical University in China began to look for useful anticancer drugs in Chinese traditional medicine. This traditional medicine bases treatment on the following principle: "use a toxic agent against something toxic." According to this principle, the cancer must be considered as something toxic (malignant) and therefore must

be treated with a toxic agent. The researchers treated some cancers with white arsenic, a well-known toxic agent, and encouraging results were observed in certain malignancies, such as carcinoma of the esophagus, malignant lymphoma, and some leukemias, particularly chronic myelogenous leukemia and APL. They found that this agent was mostly effective in the treatment of APL. Therefore, they prepared a pure solution of As₂O₃ for clinical use. The efficacy of As₂O₃ in the treatment of APL has been confirmed in China and in western countries not only for newly diagnosed APL, but also for relapsed cases. Arsenic sulfide also proved effective in the treatment of chronic myelogenous leukemia and APL.

2. Clinical efficacy in the treatment for remission induction

In 1992⁽⁴⁾, the Harbin Study Group reported that of 32 APL patients treated with A1-1 (antileukemic agent number 1, consisted of 1% white arsenic solution), 21 (65.6%) entered into CR; the 5-year survival rate was 50%, and the 10-year survival rate was 18.8%. In 1995, Huang et al⁽⁵⁾ reported that 60 APL patients treated with a compound of Realgar named Composite Indigo Naturalis tablets (containing arsenic sulfide, baphicacanthus cusia, radix salviae miltiorrhizae, radix pseudostellariae) in combination or not with mild chemotherapy; 59 (98%) achieved CR. The following year, in 1996⁽⁶⁾, a pure 1% As₂O₃ solution was used in clinical trials which demonstrated that 22/30 (73.3%) of novel APL and 22/42 (52.4%) of relapsed or refractory cases achieved CR. The above results were confirmed by other institutions in China, including our Institute⁽⁷⁾ and by the Memorial Sloan-Kettering Cancer Institute in the US,⁽⁸⁾ where 11 out of 12 (92%) of the APL patients in relapse achieved CR. Recently, the Shanghai Cooperative Group has conducted a study on the use of As₂O₃ solution in remission induction treatment of 11 novel and 47 relapsed APL (data to be published); the CR rates were 72.7% (8/11) and 85.1% (40/47), respectively (**Table 1**).

2. Mode of administration

The dose of As₂O₃ (1% solution) for adults was 10 mg/day in 250–500 ml of 5% glucose saline and administered by intravenous drip for 2–3 hours. The duration of one course of treatment was 28–48 (median 34) days. Chemotherapy or ATRA was added according to WBC and response to the treatment. Thirty-eight patients were treated by As₂O₃ alone; chemotherapy was added in 15 patients with hydroxyurea or DA protocol as the WBC was 10<10⁹/L. In five patients, ATRA was added because no marked response or even de-

Table 1. Results of the treatment of APL with As₂O₃.

Newly Diagnosed	Relapsed	
Number	11	47
Sex (M/F)	3/8	29/18
Mean age (range)	41 (24-60)	38 (7-55)
t(15;17) positive	9/11	19/22*
Protocols for the treatment:		
As ₂ O ₃ alone	7	31
+ Chemotherapy	4	11
+ATRA		5
CR	72.7% (8/11)	85.1% (40/47)
Days to CR (median)	35	31
Total dosages (mg)	295	310

teriation was observed, evidenced by elevation of promyelocytes in the peripheral blood or bone marrow picture on day 18–24 of the treatment.

In the majority of the cases (n=48), one course of treatment sufficed for achieving CR. Patients were considered treatment failures if they showed no response after two courses separated by an interval of one week. Interestingly, among the three nonresponders, one had mixed karyotype with PML/RAR α and AML1-ETO, and in the second patient, PML/RAR α fusion gene was negative, while it had been positive at his first presentation. Days to CR were 31 and 35 respectively.

3. Hemostatic Parameters during the As₂O₃ Treatment

As observed in ATRA treatment, administration of As₂O₃ did not aggravate hemorrhage of the disease; on the contrary, the bleeding related to DIC was ameliorated in accordance with correction of abnormal hemostatic parameters after treatment, as evidenced by (1) decrease of platelet granule membrane protein-140 (GMP-140), soluble fibrin monomer complex (SFMC), D-D dimer, increase of fibrinogen in the circulation, reflecting amelioration of DIC, which frequently occurs in this disease leading to hemorrhagic diathesis; (2) decline of tissue factor (TF) antigen and mRNA, as well as membrane procoagulant activity (PCA) on APL cells, indicating that As₂O₃ can downregulate the production of TF at the protein and mRNA levels and PCA activity on leukemic cells. It is well known that these two factors play an important role in the genesis of DIC in APL. Surprisingly, the level of TF antigen in circulation was not affected, even slightly elevated during the arsenic treatment. It is conceivable that decrease of these two procoagulant factors induced by the As₂O₃ on the leukemic cells was the major mechanism of reversal of hemorrhagic manifestations. Interestingly, in vitro study demonstrated that treatment of NB4 cells with As₂O₃ resulted the raise of TF and PCA on the cells, while treatment with daunorubicin was able to elevate production of TF antigen, its mRNA and PCA, which

Table 2. Adverse effects of As₂O₃.

Adverse effects	de novo APL n (%)	relapses APL n (%)	p value
Skin reaction	2 (18.1)	12 (25.5)	NS
GI disturbances	4 (36.4)	10 (21.3)	NS
Liver function damage	7 (63.6)	15 (31.9)	<0.05
Arrhythmia, A-V block	1 (9)	8 (17)	NS
Hyperleukocytosis	8 (72.7)	26 (55)	NS
ARDS	0	1 (2.1)	NS
Numbness	0	2 (4.2)	NS
Death probably due to arsenic toxicity	2 (18.2)	0	<0.05

correlates with the fact that daunorubicin can aggravate the bleeding diathesis during the treatment.

4. Adverse Effects

The major adverse and toxic effects of As₂O₃ are listed in **Table 2**. Usually they subsided spontaneously even with continuation of therapy; in a few cases, a temporary dosage decrease or suspension of the treatment was necessary. No death occurred in relapsed APL, while in 2/11 de novo cases, severe liver damage accompanied by jaundice was noted.

5. Post-remission Treatment

As in ATRA treatment, As₂O₃ therapy cannot eradicate the leukemic clone. Molecular monitoring revealed that 18 of 20 APL patients still had PML/RAR α fusion gene after CR induced by As₂O₃ therefore, post-remission treatment was necessary.

Protocols for the post-remission treatment are divided into three types: (1) Protocol A: chemotherapy alone with DA (daunorubicin and arabinoside) and MA (mitoxantrone and arabinoside) alternatively; (2) Protocol B: As₂O₃ alone for 2–3 courses. Each course consisted of As₂O₃ at a dose of 10 mg/day for 28 days, with a one-month interval between courses; (3) Protocol C: a combination of these two protocols. In 33 patients in relapsed APL followed after CR for 1–42 months (median 25 months), relapse occurred in 3/4 of group A, in 12/18 of group B, and in 2/11 of group C. The actual median overall survival (OS) duration was 25 months; the estimated DFS rates at one and two years were 63.6% and 41.6%, respectively, and the median DFS was 17 months. It therefore seemed that use of As₂O₃ combined with chemotherapy could achieve a longer survival duration. The eight newly diagnosed patients were followed after CR for 8 to 20 (median 12) months, and no recurrence was observed up to the observation period (**Table 3**).

6. Studies on the Mechanism of Action of As₂O₃

A large number of investigations were performed to clarify the mechanism of the action of As₂O₃. The results can be summarized as follows:

(1) In vitro study demonstrated that As_2O_3 possessed a dual mechanism of action⁽⁹⁾, at a lower concentration (0.1–0.5mmol/L), it was able to trigger differentiation, while at higher concentrations (0.5–2.0mmol/L), it could induce apoptosis of NB4 cells; (2) As_2O_3 at lower or higher concentration was capable of degrading PML/RAR α protein, which is known to have the property of inhibiting differentiation of promyelocytes; (3) at high concentrations, its activity to induce apoptosis of NB4 cells was correlated with the collapse of mitochondrial transmembrane potential (DYm), evidenced by negative propidium iodide (PI, a membrane-impermeable DNA-binding dye) and low rhodamine 123 (Rh123, a cationic lipophilic fluorochrome taken up by mitochondria in proportion to the DYm) labelling; (4) activation of caspase 3 (effector enzyme of apoptosis); (5) As_2O_3 was able to downregulate Bcl-2 gene expression, a gene known as an inhibitor of apoptosis, no alteration of c-myc, bax, bcl_{LX} and p53 genes was observed. Importantly, the disruption of DYm and activation of caspase 3 were antagonized by a disulfide bond reducing agent, dithiothreitol (DTT), and promoted by a glutathione (GSH) synthesis inhibitor, buthionine sulfoximide (BSO), indicating therefore that the SH group was involved in the mechanism of action of As_2O_3 .

Conclusion

As_2O_3 alone can achieve a high CR in newly diagnosed or relapsed APL. At concentrations used for treatment, As_2O_3 exerts its action by a mechanism different from that of ATRA and chemotherapy, essentially by inducing apoptosis. At lower concentration, As_2O_3 possesses the activity to induce differentiation of APL cells. Therefore, a rational protocol composed of these three agents in the treatment of APL should yield a higher CR rate as well as a longer survival duration, even cure of the disease. Presumably this could provide a model of a combination of distinct agents with different mechanisms of action, one that induces differentiation, a second that triggers apoptosis, and a third that kills malignant cells.

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