Delayed Recovery of Normal Hematopoiesis in Arsenic Trioxide Treatment of Acute Promyelocytic Leukemia: A Comparison to All-*trans* Retinoic Acid Treatment

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Abstract

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Objective To examine laboratory data including total blood cell count, leukocyte morphology and coagulation parameters during treatment for acute promyelocytic leukemia (APL) at a single institute, and compare the precise differences between all-*trans* retinoic acid (ATRA) and arsenic trioxide (As₂O₃) treatment.

Patients and Methods Sixteen patients with APL who were treated with ATRA or As₂O₃ alone and achieved complete remission (CR) were analyzed. ATRA 45 mg/m²/day was given orally until CR. As₂O₃ 0.15 mg/kg/day was given intravenously until leukemic blasts and promyelocytes were eliminated from the bone marrow.

Results All 7 patients in the ATRA-treated group were primary cases and all 9 patients in the As₂O₃-treated group were relapsed cases after the achievement of CR with the ATRA. There was no difference in the data before treatment between these two groups. The duration of leukocytopenia and neutropenia during As₂O₃ treatment was significantly longer than those of ATRA treatment. The nadir of leukocyte and neutrophil counts was observed later in the As₂O₃-treated group. Terminal neutrophil differentiation was observed more obviously in the ATRA-treated group. The red blood cell count and hemoglobin concentration decreased significantly at the end of As₂O₃ treatment and were lower than those of ATRA treatment. Platelets recovered earlier in the ATRA-treated group. Coagulation parameters were not significantly changed between the two groups.

Conclusion In comparison with ATRA treatment, the recovery of several components in the peripheral blood cells was delayed in As₂O₃ treatment. Therefore we should pay more and longer attention in As₂O₃ treatment.

Key words: laboratory data, acute promyelocytic leukemia, all-*trans* retinoic acid, arsenic trioxide

Introduction

High complete remission (CR) rate for acute promyelocytic leukemia (APL) patients has been shown in many clinical studies, and all-*trans* retinoic acid (ATRA) is now used as the first-line drug for inducing CR in APL patients (1–7). Recently, another promising agent, arsenic trioxide (As₂O₃) has been introduced for refractory and relapsed APL (8), and a high CR rate in both ATRA-resistant and ATRA-sensitive APL patients has been reported (9–13).

There are some similarities and some differences in the mechanisms of action for APL between ATRA and As₂O₃. ATRA has been used as a potent inducer of differentiation in the treatment of APL and is proven to induce APL cells to terminally differentiate along a neutrophilic pathway both *in vivo* and *in vitro* (1–3). ATRA specifically targets the aberrant fusion receptor PML-RAR α , and ATRA-induced differentiation of APL cells results in degradation of PML-RAR α fusion oncoprotein and the relocation of wild-type PML (14, 15). On the other hand, As₂O₃ has dose-dependent dual effects in APL cells: in an *in vitro* study (16), it induced apoptosis at a relatively high concentration and induced partial differentiation at a relatively low concentration. When used *in vivo*, induction of differentiation and apoptosis in leukemic cells is seen (17, 18). As₂O₃ also causes degradation and modification of PML-RAR α protein in a different way than ATRA at least in part (14, 15, 19, 20), and affects

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numerous cellular signaling pathways associated with cell growth, apoptosis and differentiation (15, 19–21).

Now, we can select the treatment for APL among ATRA, As₂O₃ and the combination of the two. In this study, we examined the laboratory data during the treatment for APL at a single institute, and compared the precise differences between ATRA and As₂O₃ treatment.

For editorial comment, see p 775.

Patients and Methods

Patients

Newly diagnosed and relapsed patients with APL in our hospital, who were treated with ATRA or As₂O₃ alone as remission induction therapy and achieved CR from April 1993 to March 2001, were included in this study. All clinical data were collected retrospectively by reviewing patient's charts. Seven patients with APL, who were newly diagnosed and achieved CR with ATRA without any other chemotherapy, were analyzed as an ATRA-treated group. In this group, ATRA 45 mg/m²/day was given orally in three divided doses after meals until CR according to AML-92 protocol of Japan Adult Leukemia Study Group (5–7). In ATRA treatment, according to the protocol, patients with an initial leukocyte count $\geq 3 \times 10^9/l$ received chemotherapy combined with the same dosage of ATRA, patients who showed blast and promyelocyte counts in peripheral blood greater than $1 \times 10^9/l$ during treatment with ATRA also received chemotherapy, and these patients were excluded in this study. The percentage of patients who received chemotherapy combined with ATRA was 46% and the CR rate of ATRA treatment in primary APL cases was 100% in our institution. Retinoic acid syndrome (RAS) was seen in 8% of the patients and hyperleukocytosis was seen in 31% of the patients. Other toxicities associated with ATRA treatment were hypertriglycemia, dry skin, stomatitis and liver dysfunction.

On the other hand, 9 cases who achieved CR with As₂O₃ without chemotherapy were analyzed as an As₂O₃-treated group. In this group, all patients had previously achieved CR with ATRA therapy with or without chemotherapy, subsequently relapsed from the CR. As₂O₃ 0.15 mg/kg/day was given intravenously over 2 hours diluted in 500 ml of 5% dextrose until leukemic blasts and promyelocytes were eliminated from the bone marrow and the residual blast count was no more than 5% of marrow mononuclear cells according to the same protocol used in the phase II study in the USA (11). In As₂O₃ treatment, some of the patients who showed leukocytosis received chemotherapy to prevent RAS-like syndrome, and they were excluded from this study. The percentage of patients who received chemotherapy combined with As₂O₃ was 21%. The CR rate of As₂O₃ treatment was 78%. As described previously, the major adverse effects of As₂O₃ were electrocardiogram abnormalities (98%) including QTc prolongation (98%), ventricular premature contraction

(57%), non-sustained ventricular tachycardia (27%) and paroxysmal supraventricular tachycardia (14%). RAS-like syndrome (7%) and leukocytosis (36%) were also seen. Other adverse effects were peripheral neuropathy, liver dysfunction, fluid retention, puritus and gastrointestinal symptoms such as nausea and vomiting (12).

Fresh-frozen plasma and platelet transfusions were given to avoid life-threatening bleeding when necessary. No life-threatening organ bleeding was seen with either treatment. Gabexate mesilate was administered in the cases with disseminated intravascular coagulopathy (DIC). All patients had stayed in hospital while receiving each therapy. Informed consent was obtained from all patients in both treatments. The diagnosis of APL was confirmed in all patients by the presence of t(15;17) and PML-RAR α fusion transcript.

The main clinical and hematological characteristics of patients are shown in Table 1. There was no statistical difference between ATRA- and As₂O₃-treated groups in gender, age, percentages of APL cells in bone marrow, total blood cell counts, leukocyte morphology and coagulation parameters before treatment. The median days of ATRA- and As₂O₃- administration were 48 (range: 39–60) and 43 (27–54), respectively, and no statistical difference was observed (Table 2). CR was defined as less than 5% of blasts in cellular bone marrow aspirate, and more than $1.0 \times 10^9/l$ neutrophils and $100 \times 10^9/l$ platelets in the peripheral blood.

Laboratory parameters

Total blood cell counts including basic leukocyte morphology and reticulocyte count were analyzed by SE9000 and R3000 (Sysmex, Kobe, Japan) every other day. Coagulation parameters including prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FBG) and fibrinogen degradation products D-dimer (FDP-DD) were examined by CA6000 and LPIA200 (Sysmex, Kobe, Japan).

Statistical analysis

The unpaired *t*-test and χ^2 test were applied for comparisons of clinical data between ATRA- and As₂O₃-treated groups using SAS computer program (SAS Institute Inc, Cary, NC). The paired *t*-test was applied for comparisons of data between before and the end of treatment in each patient.

Results

Leukocyte count and morphology

The time courses of leukocyte and neutrophil (segmented and band forms) counts during treatments are shown in Fig. 1. The leukocyte and neutrophil counts at the end of As₂O₃ treatment were significantly lower than those at the end of ATRA treatment (Table 2), and the durations of leukocytopenia ($\leq 1.0 \times 10^9/l$) and neutropenia ($\leq 0.5 \times 10^9/l$) in the As₂O₃-treated group were significantly longer than those in the ATRA-treated group (Table 3). Although the lowest counts of leukocytes and neutrophils were not significantly

Table 1. Characteristics and Clinical Data of APL Patients before ATRA or As₂O₃ treatment

	ATRA	As ₂ O ₃	P value
Number of patients	7	9	
Male/Female	4/3	4/5	NS
Status of disease			
Primary/Relapsed	7/0	0/9	<0.001
Age (years)*	55 (21–81)	43 (23–61)	NS
APL cells in bone marrow (%)*	87 (36–92)	72 (16–94)	NS
Leucocyte (×10 ⁹ /l)*	0.70 (0.50–2.70)	1.00 (0.30–7.80)	NS
Blast+Promyelocyte (×10 ⁹ /l)*	0.04 (0–1.30)	0 (0–3.20)	NS
Myelocyte+Metamyelocyte (×10 ⁹ /l)*	0.01 (0–0.03)	0 (0–0.08)	NS
Neutrophil (×10 ⁹ /l)*	0.20 (0.35–0.50)	0.58 (0–1.07)	NS
RBC (×10 ⁹ /l)*	2,820 (1,370–4,280)	3,460 (2,460–4,140)	NS
Hemoglobin (g/dl)*	8.7 (4.3–14.7)	11.2 (7.5–13.3)	NS
Reticulocyte (×10 ⁹ /l)*	38 (30–135)	53 (3–121)	NS
Platelet (×10 ⁹ /l)*	34 (12–144)	82 (42–202)	NS
PT (%)*	73 (12–102)	87 (59–106)	NS
APTT (%)*	109 (35–140)	89 (56–140)	NS
FBG (mg/dl)*	184 (50–350)	245 (90–449)	NS
FDP-DD (μg/ml)*	18 (1.4–82.9)	27.4 (4.5–72.8)	NS

*Data are shown as median (range). NS denotes not significant. APL: acute promyelocytic leukemia, ATRA: all-*trans* retinoic acid, As₂O₃: arsenic trioxide, RBC: red blood cell, PT: prothrombin time, APTT: activated partial thromboplastin time, FBG: fibrinogen, FDP-DD: fibrinogen degradation products D-dimer.

Table 2. Clinical Data of APL Patients at the End of ATRA or As₂O₃ Treatment

	ATRA (n=7)	As ₂ O ₃ (n=9)	P value
Days of administration	48 (39–60)	43 (27–54)	NS
Leukocyte (×10 ⁹ /l)	3.4 (1.5–4.0)	0.9 (0.3–3.0)	<0.001
Blast+Promyelocyte (×10 ⁹ /l)	0 (0–0)	0 (0–0)	NS
Myelocyte+Metamyelocyte (×10 ⁹ /l)	0 (0–0.07)	0 (0–0.01)	NS
Neutrophil (×10 ⁹ /l)	1.37 (1.23–2.00)	0.19 (0.05–0.69)	<0.001
RBC (×10 ⁹ /l)	3,400 (2,610–4,060)	2,560 (1,920–2,990)	<0.01
Hemoglobin (g/dl)	10.4 (8.4–13.0)	8.1 (6.3–10.4)	<0.05
Reticulocyte (×10 ⁹ /l)	101 (62–241)	79 (17–219)	NS
Platelet (×10 ⁹ /l)	308 (198–503)	86 (15–381)	<0.01
PT (%)	85 (70–102)	99 (82–123)	<0.05
APTT (%)	78 (66–102)	69 (34–124)	NS
FBG (mg/dl)	288 (224–361)	288 (196–587)	NS
FDP-DD (μg/ml)	2 (0–4.4)	0 (0–5.2)	NS

Data are shown as median (range). NS denotes not significant. APL: acute promyelocytic leukemia, ATRA: all-*trans* retinoic acid, As₂O₃: arsenic trioxide, RBC: red blood cell, PT: prothrombin time, APTT: activated partial thromboplastin time, FBG: fibrinogen, FDP-DD: fibrinogen degradation products D-dimer.

different between ATRA- and As₂O₃-treated groups, their nadirs in the As₂O₃-treated group were significantly later than those in the ATRA-treated group (Table 3), and the recovery of leukocyte and neutrophil counts was delayed in the As₂O₃-treated group.

As for the time courses of blast, promyelocyte, myelocyte and metamyelocyte counts during treatment, the blasts and promyelocytes had appeared in peripheral blood during a similar period (around 10 days) in both groups. The highest

count was not significantly different between the two groups, but their peak in the As₂O₃-treated group (median, 10.5 days; range, 1–24) was later than that in the ATRA-treated group (median, 1 days; range, 1–5). On the other hand, no significant difference was observed as for the myelocyte and metamyelocyte counts in peripheral blood during therapy between the two groups.

Figure 1. Time courses of clinical laboratory data from APL patients who achieved CR by ATRA or As₂O₃ treatment. APL: acute promyelocytic leukemia, ATRA: all-*trans* retinoic acid, As₂O₃: arsenic trioxide, PT: prothrombin time, FDP-DD: fibrinogen degradation products D-dimer.

Table 3. Clinical Data of APL Patients during ATRA or As₂O₃ Treatment

	Nadir (days after the start of treatment)		P value
	ATRA	As ₂ O ₃	
Leukocyte	6.0±9.8	20.7±14.8	<0.05
Neutrophil	2.3±1.4	25.3±12.1	<0.001
RBC	19.7±15.3	32.2±8.8	<0.05
Hemoglobin	17.0±15.6	31.0±9.0	<0.05
Platelet	5.0±6.8	26.3±10.7	<0.001

	Duration (days)		P value
	ATRA	As ₂ O ₃	
Leukocytopenia ($\leq 1 \times 10^9/l$)	7.7±9.7	24.1±18.3	<0.05
Neutropenia ($\leq 0.5 \times 10^9/l$)	20.0±10.9	35.0±11.5	<0.05
Thrombocytopenia ($\leq 50 \times 10^9/l$)	8.1±11.5	22.4±14.4	<0.05

The data are shown with mean±SD. NS denotes not significant. APL: acute promyelocytic leukemia, ATRA: all-*trans* retinoic acid, As₂O₃: arsenic trioxide, RBC: red blood cell.

Red blood cell

The red blood cell (RBC) count and hemoglobin concentration at the end of As₂O₃ treatment were significantly lower than those of the ATRA-treated group (Table 2). In the ATRA-treated group, the RBC count and hemoglobin concentration at the end of treatment increased more than those before treatment in six of seven patients. On the other hand, in the As₂O₃-treated group, the RBC count and hemoglobin concentration decreased in eight of nine patients at the end of treatment less than those before treatment. The nadirs of RBC count and hemoglobin concentration in the As₂O₃-treated group were significantly later than that in the ATRA-treated group (Table 3).

The reticulocyte count during treatment was not significantly different between the two groups.

Platelet

The platelet count at the end of As₂O₃ treatment was significantly lower than that at the end of ATRA treatment (Table 2), and the duration of thrombocytopenia ($\leq 50 \times 10^9/l$) in the As₂O₃-treated group was significantly longer than that in the ATRA-treated group (Table 3). Although the lowest platelet count did not differ between the two groups, the nadir of the platelet count in the As₂O₃-treated group was significantly later than that in the ATRA-treated group (Table 3). In addition, although the platelet count at the end of ATRA treatment increased more than that before treatment and reached normal range in all patients, the platelet count did not increase significantly and remained at less than $100 \times 10^9/l$ in five of nine patients at the end of As₂O₃ treatment.

Coagulation parameter

The time courses of PT and FDP-DD are shown in Fig. 1. Within the first week after the start of treatment, the bleeding symptoms were rapidly improved in all patients. PT was shortened at the end of therapy in all patients who showed

prolonged PT before treatment and it was normalized in all patients at the end of treatment. APTT (%) changed variably from patient to patient and no significant difference was observed between ATRA- and As₂O₃-treated groups. FBG concentration increased at a relatively early stage of treatment and reached the normal range in all patients who showed a low FBG concentration before treatment. While FDP-DD was elevated in all patients before treatment, it was rapidly reduced after the start of ATRA or As₂O₃ treatment. Coagulation parameters examined did not change significantly between ATRA- and As₂O₃-treated groups.

Discussion

In this study, all 16 patients obtained CR by ATRA or As₂O₃ without combined chemotherapy. All 7 patients in the ATRA-treated group were primary cases and all 9 patients in the As₂O₃-treated group were relapsed cases after the achievement of a CR with the ATRA. However, no statistical difference was observed in their clinical characteristics and data before treatment between these two groups and there was also no difference in the treatment period between these two groups. In previous reports, the time to obtained CR in relapsed/refractory APL patients treated with ATRA was the same as that in newly diagnosed APL patients and no significant difference was observed also in the As₂O₃-treated APL patients between the time to obtained CR in relapsed/refractory cases and that in newly diagnosed cases (4, 9). Even if any of the effects of additional hematopoietic disorder or cytogenetic change in the relapsed cases for the laboratory data of the As₂O₃-treated group are undeniable, it is still important to know the difference in laboratory data between these two groups, because, in the present status, ATRA is generally used as the first choice drug for newly diagnosed APL and As₂O₃ as the treatment for ATRA-resistant or relapsed APL.

It is reported that the increase in platelets was the most prominent after 3 weeks and elevation of the hemoglobin concentration appeared reluctant and slow in ATRA treatment (1), and that the mean time of normalization in ATRA treatment was day 30 for thrombocytopenia, day 50 for neutropenia, and day 60 for anemia (2). In addition, ATRA induces terminal differentiation of APL cells (1–3). On the other hand, in As₂O₃ treatment, significant changes of hemoglobin and platelet levels were not observed in an earlier report (8), and neutropenia is reported as an adverse effect of As₂O₃ treatment (12). It is also reported that the differentiating effect of As₂O₃ for APL cells is incomplete and terminally differentiated cells such as polynuclear granulocytes do not increase in As₂O₃ treatment (17, 22). However, in these reports, the clinical laboratory findings of ATRA and As₂O₃ treatments have been described separately and the differences between these two treatments have been not clearly demonstrated. In the present study, by comparing the time course of blood cell components and coagulation parameters during ATRA and As₂O₃ treatment without chemotherapy, we confirmed the previous observation and could clearly demonstrate the difference between these two treatments.

Examination of total peripheral blood cell counts, and the duration of severe leukocytopenia ($\leq 1 \times 10^9/l$) and neutropenia ($\leq 0.5 \times 10^9/l$) during As₂O₃ treatment were significantly longer than that of ATRA treatment. The nadir of leukocyte and neutrophil counts in the As₂O₃-treated group was observed later than that in the ATRA-treated group. In addition, the leukocyte and neutrophil counts remained low at the end of therapy in the As₂O₃-treated group and terminal neutrophil differentiation was more marked in the ATRA-treated group. As for the RBC count and hemoglobin concentration, the recovery started in the early stage of the ATRA-treated group, but it was not observed in the As₂O₃-treated group. The platelet recovery was also found in the early stage of the ATRA-treated group, but thrombocytopenia in the As₂O₃-treated group continued during the therapy and the duration of severe thrombocytopenia in As₂O₃ treatment was significantly longer than in ATRA treatment. Thus, in ATRA treatment, the recovery of normal blood cell components starts in the early stage of therapy, but in As₂O₃ treatment, it is not always observed and cytopenia often continues during therapy. On the other hand, recently Shen et al described ATRA and As₂O₃ combined therapy compared with ATRA or As₂O₃ mono-therapy in primary APL. They showed that whole peripheral blood cell counts during each therapy did not change significantly between ATRA- and As₂O₃-treated groups (23). Their subjects included chemotherapy-treated patients (a relatively high percentage around 60%) and the daily dosage of ATRA was relatively low (25 mg/m²). Although it is difficult to simply compare their data to ours, combined chemotherapy or ATRA dosage could affect the time course of peripheral blood cell counts during therapy in APL. In addition, the status of patients (primary/relapse) might affect them.

On the other hand, coagulation parameters, particularly

FDP-DD, were rapidly improved after the start of therapy in both treatments. The mechanisms of correction for coagulopathy in APL by ATRA have been studied extensively, however those by As₂O₃ have remained unclear. Zhao et al reported a different behavior in thrombomodulin and tissue plasminogen activator levels during therapy between ATRA and As₂O₃ treatment (24). It may suggest that the mechanism of correction of coagulopathy in APL was different between the two. However, they also reported that, in both therapies, there was no significant difference in other important hematopoietic parameters concerning platelet activation, natural anticoagulants and fibrinolysis proteins. In the present study, no significant difference was observed clinically in PT, APTT, FBG concentration and FDP-DD concentration between ATRA and As₂O₃ treatment. Therefore, we infer that the clinical status of coagulopathy and fibrinolysis during therapy is not significantly different between these two treatments. In either treatment, rapid improvement of coagulation parameters is observed after the start of therapy, so it is important for APL to start these treatments as soon as possible in order to avoid life-threatening hemorrhaging.

In conclusion, we could clarify that the recovery of several components in peripheral blood cells was delayed in As₂O₃ treatment. The difference of laboratory data between the two treatments should be noted and it is necessary to pay more attention for a longer period with As₂O₃ treatment.

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