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Pharmacokinetics of All-*trans* Retinoic Acid in Pediatric Patients with Leukemia

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Since all-*trans* retinoic acid (ATRA) induces complete remission in a high proportion of patients with acute promyelocytic leukemia (APL), and its effectiveness appears to be related to the plasma or serum level, a pharmacokinetic study of ATRA was undertaken in nine patients with various leukemias. After oral administration at a dose of 30 mg/m^2, the time required to reach the peak plasma level of ATRA (20–1198 ng/ml) was between 120 and 240 min and the apparent plasma elimination half life was 21–51 min. In addition, 13-*cis* retinoic acid was detected in the plasma of seven patients, indicating the occurrence of ATRA isomerization *in vivo*. ATRA therapy did not induce complete remission in all patients, even when high plasma levels were achieved. Among the six APL patients given ATRA therapy, one who failed to respond had a very low plasma ATRA level. These findings suggest that it may be useful to monitor plasma levels during oral ATRA therapy in order to achieve an appropriate treatment regimen.

Key words: Retinoic acid — Acute promyelocytic leukemia — Pharmacokinetics

All-*trans* retinoic acid (ATRA) is an active metabolite of vitamin A which is known to play a critical physiologic role in tissue development and cell differentiation.[1,2] Breitman *et al.*[3] showed that ATRA induces differentiation of the HL-60 cell line derived from human acute myelocytic leukemia. In 1988, a Chinese group[4] orally administered ATRA to patients with acute promyelocytic leukemia (APL) and reported a high rate of initial remission induction. A similar clinically beneficial effect of ATRA therapy was confirmed by other research groups,[5,6] and it was reported that 80–90% of untreated or relapsed APL patients could achieve complete remission after ATRA therapy.[7] The initial clinical response to ATRA is most closely correlated with a chromosome anomaly, the t(15;17) translocation, which is known to be pathognomonic for APL and is associated with the promyelocytic leukemia (PML)/retinoic acid receptor (RAR)-α fusion protein.[8] Clinical and molecular studies have suggested that the product of this fusion gene may serve as a molecular target for ATRA therapy.

However, continuous therapy with ATRA can lead to the development of resistance or relapse problems,[4–6] which may be related to its pharmacokinetics. So far, there have been very few reports on the pharmacokinetics and therapeutic effect of ATRA in pediatric APL patients. In the present study, plasma ATRA levels were measured in pediatric patients with APL and other hematologic diseases, and the efficacy of ATRA therapy was evaluated.

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MATERIALS AND METHODS

Subjects The clinical characteristics of the six pediatric patients with APL, two with acute myelocytic leukemia (AML) and one with myelodysplastic syndrome (MDS) who received ATRA therapy are listed in Table I. A diagnosis of acute leukemia was made on the basis of the French-American-British (FAB) classification. Among the six children with APL, three were in relapse (patient Nos. 1, 4, and 6) and the other three (patient Nos. 2, 3, and 5) were untreated. The patients with other diseases were also untreated until ATRA therapy was initiated.

ATRA administration and sample collection ATRA tablets were kindly provided by the Shanghai Second Medical University; each tablet contained 6.6 mg of ATRA (although the inscription read 10 mg), and the ATRA content was confirmed to be chemically pure all-*trans* retinoic acid without any isomers. Without chemotherapy using anticancer drugs, a single oral dose of ATRA (20 mg/m^2 for patients No. 1 and 30 mg/m^2 for the other eight patients) was given in the morning after breakfast. In seven patients, the pharmacokinetics were investigated on day one of ATRA therapy and three of these seven patients (Nos. 5, 7, and 8) were studied again after 2–6 weeks of ATRA therapy. Another two patients (Nos. 1 and 4) underwent first investigation after 21 months of therapy. Blood samples were collected via venipuncture into heparinized tubes before drug administration, and were centrifuged at 3,000 rpm for 10 min to provide plasma, which was stored in the dark at −80°C until analysis. The study protocol was approved

Table I. Clinical and Hematological Characteristics of 9 Patients Receiving Oral ATRA

Patient No.	Disease	Sex	Age	Dose of ATRA (mg/m^2)	t(15;17)	RAR-α	PML	Outcome
1	AML3	F	18	20	positive	NT	NT	CR
2	AML3	F	12	30	positive	NT	NT	CR
3	AML3	M	15	30	positive	positive	positive	CR
4	AML3	M	15	30	positive	NT	positive	CR
5	AML3	M	6	30	positive	positive	positive	CR
6	AML3	M	13	30	positive	positive	positive	failure
7	MDS	M	10	30		NT	NT	failure
8	AML4	M	4	30		NT	NT	failure
9	AML2	M	6	30		NT	NT	failure

Abbreviations: F, female; M, male; t(15;17), t(15;17) translocation; RAR-α, rearrangement of RAR-α; PML, rearrangement of PML; CR, complete remission; NT, not tested.

by the Ethics Committee of the college hospital, and informed consent to the investigation was obtained from the subjects and/or their parents.

Assay procedure A 2.5 ml aliquot of 0.1 *M* phosphate buffer (pH 6.0) and 5 ml of ethyl ether were added to 1 ml of plasma in a darkened tube. The mixture was vortexed for 20–30 s and then centrifuged at 3,000 rpm for 15 min. A 4 ml aliquot of the upper layer was removed and evaporated to dryness, after which the residue was dissolved in methanol and analyzed by high-performance liquid chromatography (HPLC).[9] The HPLC system included a 655 Liquid Chromatograph pump (Hitachi, Tokyo) and a QC pack-C18 column (Irica, Kyoto). The mobile phase consisted of 60% acetonitrile and 40% ammonium acetate buffer (v/v, 10%) at a flow rate of 1.5 ml/min. The eluate was monitored using a Spectrophotometer Σ873 (Irica, Kyoto) with detection at 340 nm. The retention times of ATRA and 13-*cis* retinoic acid were 8.4 and 8.6 min, respectively (Fig. 1). The 4-oxo all-*trans* retinoic acid peak could not be separated from other peaks, including that of 4-oxo 13-*cis* retinoic acid. The recovery rate was 90–95% for ATRA plus 13-*cis* retinoic acid and the detection limit was 10 ng/ml. Pharmacokinetic parameters were calculated for each patient. The plasma half life was estimated using a one-compartment model, and the area under the concentration-time curve (AUC) was determined by trapezoidal approximation. Authentic ATRA and 13-*cis* retinoic acid were purchased from Sigma Chemical Co. (St. Louis, MO), and the other chemicals used were obtained from Nacalai Tesque Co., Ltd. (Kyoto).

Southern blot analysis Bone marrow or peripheral blood mononuclear cells were obtained from five APL patients immediately before ATRA administration, and were purified by Ficoll-Hypaque centrifugation. Southern blot analysis for the normal RAR-α gene and PML gene was performed on total cellular DNA as previously described by de The *et al.*[10]

RESULTS

Clinical effect of ATRA The APL patients received a single dose of ATRA on the first day and then two equal doses daily for 2–4 weeks. Five out of the six patients achieved complete remission, while one patient showed no objective response. The two patients with AML and one with MDS also showed no response, but all of them achieved complete remission with conventional chemotherapy immediately after ATRA therapy. All the APL patients showed the t(15;17) translocation in their bone marrow cells before ATRA therapy. The blast cells from three APL patients showed RAR-α rearrangement, while those from four APL patients showed PML rearrangement. Both the translocation and rearrangements disappeared from the cells after successful therapy (Table I). With continuous ATRA therapy, nausea and headache developed in the majority of patients, but subsided after further administration or after suspension of treatment. Skin involvement occurred in three patients, while hypercalcemia and arrhythmia appeared in one patient (No. 4). All these symptoms disappeared immediately upon suspension of ATRA therapy. No patient treated with ATRA showed any significant abnormalities of hepatic or renal function. There was no case in which ATRA therapy had to be discontinued.

Pharmacokinetics The pharmacokinetic parameters obtained after the initial administration of ATRA on day one are summarized in Table II. Before administration, ATRA and its derivatives were not detected in the plasma of any patient. When the plasma level was measured every hour after an initial oral dose of 30 mg/m^2 in all patients (except No. 1, 20 mg/m^2), the peak level was reached after 1–4 h (mean: 154±47 min, M±SD), but rapidly decreased, becoming undetectable (< 10 ng/ml) after 6–8 h. Although changes in the ATRA level were characterized by marked interpatient variation, the mean peak plasma ATRA level was 429.7±408.7 ng/ml (M±

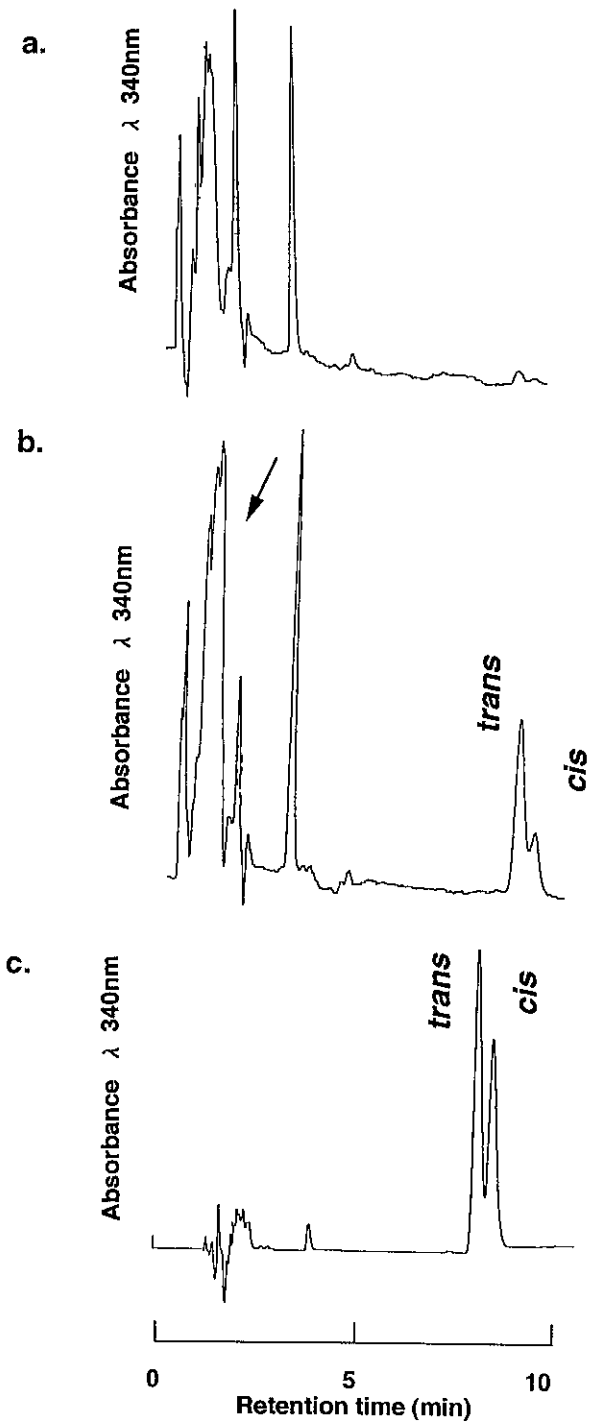

Fig. 1. a. Chromatogram of plasma obtained before ATRA administration, b. Chromatogram of plasma obtained after ATRA administration, c. Chromatogram of authentic standards; 50 ng/ml of ATRA and 13-*cis* retinoic acid. *cis* peak, 13-*cis* retinoic acid; *trans* peak, ATRA; arrow, the retention time of 4-oxo all-*trans* retinoic acid or 4-oxo 13-*cis* retinoic acid, which could not be clearly separated from other unknown peaks.

SD). The plasma ATRA level rapidly decayed monoexponentially with a harmonic half life of 30.4±8.5 min (M±SD). The AUC was calculated to be 971.9±989.7 ng/ml/h (M±SD). Fig. 2 shows changes in the mean plasma levels of ATRA in seven patients after the initial dose on day one.

In three patients (Nos. 5, 7, and 8) who were initially examined on day one and who subsequently continued ATRA therapy, a second examination was performed after 2–6 weeks. The time when the peak level was reached was later and the peak level was lower in all patients as compared with that of day one.

After ATRA administration, 13-*cis* retinoic acid was also detected in the plasma of 7 patients, reaching a maximum of 6–71 ng/ml (Table III).

The pharmacokinetics of ATRA in four patients with APL are shown in Fig. 3. Three patients who achieved complete remission showed higher plasma levels as compared with the patient (No. 6) who failed to achieve remission. This patient suffered a second relapse after conventional chemotherapy. At this time, a pharmacokinetic study was again performed on the first day of repeat ATRA therapy, and also showed a lower plasma level of ATRA (Table II). This patient did not achieve remission with the repeat ATRA therapy either.

One patient (No. 5) achieved complete remission which was maintained for a long period (8 months) after six weeks of ATRA therapy followed by combination chemotherapy performed intermittently for one week every month and additional ATRA for two weeks in the next month (see schedule in Fig. 4). During the course of treatment, a pharmacokinetic study of ATRA was carried out every 2–3 months on the first day of repeat ATRA therapy. A total of four studies were done as indicated by the arrows in Fig. 4. The peak plasma level of ATRA was decreased at the second and third examinations, while it increased again at the fourth examination to approximate that seen at the first examination (741 vs. 540 ng/ml) (Fig. 5).

DISCUSSION

On the basis of a previous report by a Chinese group,[4] a dose of 45 mg/m^2 of ATRA was scheduled for administration in this study. However, since the ATRA tablet preparation which was kindly provided by the Shanghai Second Medical University, contained 6.6 mg ATRA per tablet (although 10 mg was inscribed on the face), the actual dose of ATRA delivered was 30 mg/m^2 in this study (except patient No. 1, 20 mg/m^2). Five of our six pediatric patients with APL achieved complete remission after ATRA therapy, but one patient failed to achieve remission. Therefore, a net ATRA dosage of 30 mg/m^2 appeared sufficient to achieve clinical benefit, since the re-

Table II. Pharmacokinetics Model-independent Parameters of ATRA in 9 Patients

Patient No.	Initial day				After 2 weeks–21 months			
	Time to peak (min)	Peak concentration (ng/ml)	$t_{1/2}$ (min)	AUC (ng/ml/h)	Time to peak (min)	Peak concentration (ng/ml)	$t_{1/2}$ (min)	AUC (ng/ml/h)
1	NT	NT	NT	NT	300	149	21	NE
2	120	443	28	903.7	NT	NT	NT	NT
3	180	205	48	488.8	NT	NT	NT	NT
4	NT	NT	NT	NT	120	529	45	1565.3
5	120	741	25	NE	180	151	41	410.7
6	240	47	34	NE	120	20	55	74.7
7	120	1198	29	2700.2	180	313	41	1728.4
8	180	185	23	386.9	180	97	28	NE
9	120	189	26	379.7	NT	NT	NT	NT
Mean±SD	154±47	429.7±408.7	30.4±8.5	971.9±989.7				

Abbreviations: NE, not estimable; NT, not tested.

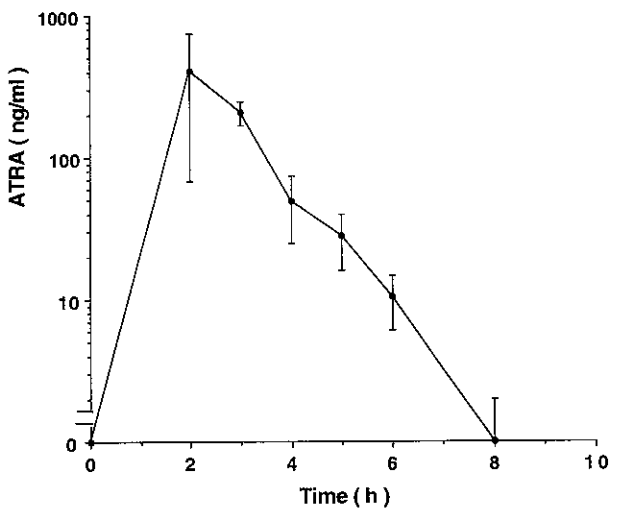

Fig. 2. Plasma levels following a single dose of ATRA (30 mg/m^2) on day one of treatment. Points, mean values; bars, SE.

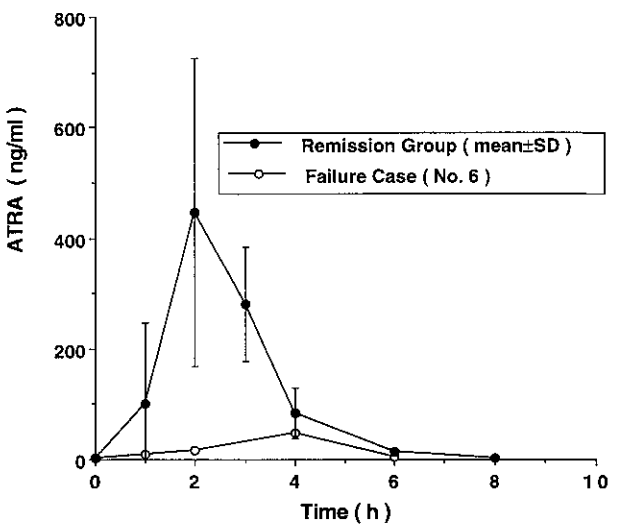

Fig. 3. Plasma concentrations of ATRA after administration of a single oral dose (30 mg/m^2) in APL patients.

Table III. Time to Peak and Maximum Concentration of 13-*cis* Retinoic Acid in Leukemia Patients Treated with Oral ATRA

Patient No.	Time to peak (min)	Peak concentration (ng/ml)
1	360	6
2	180	39
3	180	15
4	240	63
5	ND	ND
6	240	12
7	180	18
8	ND	ND
9	120	71

ND, not detected.

sponse of our pediatric APL patients was consistent with that previously reported in adult APL patients.[4–6, 11, 12] Before administration of ATRA, there was no detectable ATRA or its isomer (13-*cis* retinoic acid) in the plasma of any patient (the detection limit of our assay was 10 ng/ml). Muindi *et al.*[13] have used liquid chromatography/mass spectrometry to determine that the endogenous plasma ATRA level is about 2 ng/ml in both APL patients and healthy subjects, while those of 13-*cis* retinoic acid, 4-oxo all-*trans* retinoic acid and 4-oxo 13-*cis* retinoic acid are 1.5 ng/ml, 0.8 ng/ml, and 2–3 ng/ml, respectively. We found that the plasma ATRA level peaked 2–4 h after oral administration and disappeared rapidly with a half life of less than 60 min. This

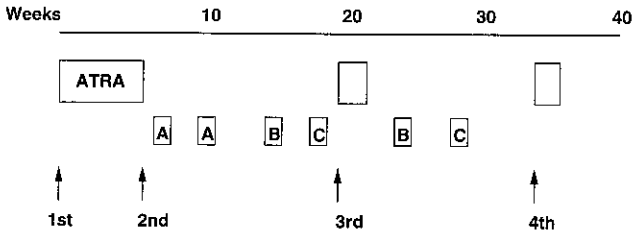

Fig. 4. Protocol for treatment and pharmacokinetic studies in patient No. 5. Arrows, ATRA pharmacokinetic studies; A, enocitabine (BA-AC), mercaptopurine (6-MP), daunorubicin, prednisolone (PDN); B, doxorubicin, cyclophosphamide, cytarabine, vincristine (VCR), 6-MP, PDN; C, BH-AC, aclarubicin, VCR, 6-MP, PDN.

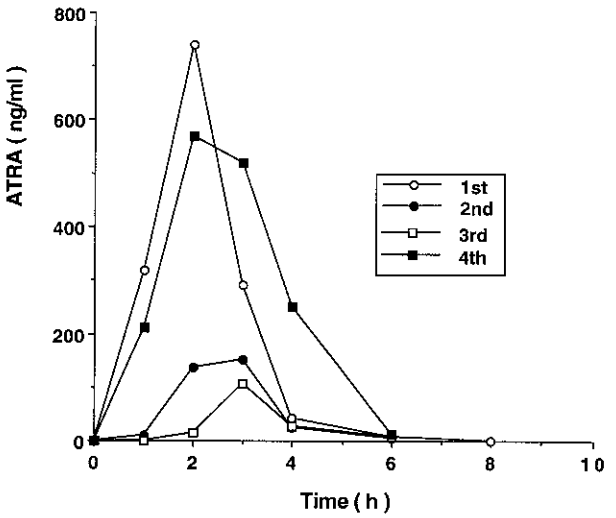

Fig. 5. Plasma concentrations of ATRA in patient No. 5 after administration of a single dose (30 mg/m^2).

pharmacokinetic pattern was in agreement with that in previous reports.[7, 13–15] However, the pharmacokinetics varied among the individual patients, and in patient No. 6, who failed to achieve remission, the plasma level differed from that in the patients achieving remission. In patient No. 6, the AUC was very low and the time to the peak level was longer as compared with the other patients. Warrell[16] investigated the effect of ATRA in APL patients and reported that some patients failed to achieve remission, and acquired resistance during continuing therapy. The acquired resistance and the individual differences in response were postulated to be due to an increase in the oxidative catabolism of ATRA[17] and individual differences in ATRA metabolism.[16] In our study, the APL patient (No. 6) who failed to achieve remission showed a low plasma ATRA level even after the first dose. Thus, failure may have been due to the fact that a sufficient ATRA concentration to achieve cytodifferentiation of leukemic cells was not achieved.

In a patient (No. 1) who showed remission for three years after receiving continuous ATRA therapy, the plasma ATRA level after 21 months of therapy still remained at a level comparable with that after the initial dose in other patients showing a response to ATRA (Table II and Fig. 3). Unfortunately, a pharmacokinetic study was not done in this patient at the start of administration. This case suggests that the plasma ATRA level may be related to maintenance of remission by ATRA monotherapy.

With respect to 13-*cis* retinoic acid, a minimal conversion of ATRA to the 13-*cis* form was detected in our study, and the metabolite had a slightly delayed peak time and prolonged half life (data not shown) as compared with those of ATRA. This confirms the findings of Muindi *et al.*,[13] who reported that the sole metabolite detected in plasma was 4-oxo all-*trans* retinoic acid, which was eliminated in the urine after glucuronide conjugation. A 4-oxo retinoic acid peak, including all-*trans* and 13-*cis* forms, was also detected in our study (data not shown). However, the behavior of the isomers could not be determined, because their analytical characteristics still remain to be confirmed.

A potentially critical finding in our investigation was that although the ATRA levels decreased with continuous administration, they remained near the initial level after intermittent administration, as shown in patient No. 5 who received combined therapy with anticancer drugs (Fig. 5). The intermittent administration of ATRA may reduce accelerated catabolism, as the ATRA level increased again at the 4th examination in this patient. Since relapse and resistance of APL to continuous treatment with ATRA are thought to be associated with progressive reduction of the plasma level,[13] the above finding indicates that intermittent ATRA therapy may reduce the development of resistance. However, further studies will be required to confirm this. In addition, our results suggest that it may be useful to monitor plasma ATRA levels during oral therapy in order to achieve an appropriate treatment regimen.

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