Rapid Paper

4-Oxo Retinoic Acid for Refractory Acute Promyelocytic Leukemia in Children with All-Trans Retinoic Acid Therapy

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Summary Therapy with all-trans retinoic acid (ATRA) achieves complete remission in a high proportion of patients with acute promyelocytic leukemia (APL), but the efficacy is reported to relate to plasma ATRA level after oral administration. The pharmacokinetics of ATRA and 4-oxo all-trans retinoic acid (4-oxo ATRA), a metabolite of ATRA, were studied in four children with APL at the time of initial oral administration. After administration of ATRA at a dose of 30mg/m², the peak plasma ATRA level was 20–741 ng/ml and was reached at 60–120 min. The patient with the lowest peak plasma level did not achieve complete remission and had a very high 4-oxo ATRA level compared to the patients with complete remission. These findings suggest that accelerated metabolism of ATRA plays a role in the failure of this agent in the patients without remission.

Key Words 4-oxo all-trans retinoic acid, all-trans retinoic acid, pharmacokinetics, acute promyelocytic leukemia

All-trans retinoic acid (ATRA), an active metabolite of vitamin A, is known to play a critical physiologic role in growth, vision, reproduction, epithelial cell differentiation, and immune function (1, 2). In 1988, a Chinese group orally administered ATRA to patients with acute promyelocytic leukemia (APL) and achieved a high rate of initial remission induction (3). The initial clinical response to ATRA is most closely correlated with a chromosome anomaly, the t(15; 17) translocation, which is known to be pathogenonomic for APL and is associated with the PML/RAR-alpha fusion protein (4). Clinical and molecular investigations have suggested that the product of this fusion gene may serve as a molecular target.

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for ATRA therapy. However, subsequent reports have shown the development of resistance to ATRA and/or leukemic relapse after a relatively short period with accelerated ATRA metabolism may be involved (4). Accordingly, we investigated plasma levels of 4-oxo ATRA (a metabolite of ATRA) and the pharmacokinetics of ATRA during the treatment of APL to assess the influence of heightened metabolism on the response to therapy.

Patients and methods

ATRA administration and sample collection. The subjects were four patients with APL diagnosed on the basis of the French-American-British (FAB) classification, who were all boys aged 6 to 15 years with the t(15;17) translocation chromosomal anomaly. Two of the four had relapse of APL and the others were untreated before ATRA therapy (Table 1). The ATRA tablets used were kindly provided by the Shanghai Second Medical University. Although 10 mg was inscribed on the face, the net ATRA content was confirmed to be 6.6 mg/tablet of chemically pure all-trans retinoic acid without any isomers or derivatives. A single 30 mg/m² dose of ATRA was administered on the first day in the morning after breakfast, and was followed by the same daily dose divided into two equal portions for the subsequent 2–4 weeks without chemotherapy using anticancer drugs. In three of the four patients (Nos. 1, 3 and 4), pharmacokinetic examination was performed on the first day of oral ATRA therapy, while in the remaining patient (No. 2) it was done later during administration.

Patient No. 4 had previously received chemotherapy and had achieved remission. When he relapsed several months later, ATRA therapy was commenced. Although no response was achieved, the plasma ATRA level was determined at the start of treatment, but 4-oxo ATRA was not assayed. Because of the failure to achieve remission, bone marrow transplantation (BMT) was performed. However, he relapsed again within two months after BMT, so induction therapy with ATRA was again attempted and pharmacokinetics were studied on the first day of treatment.

Blood samples were collected via venipuncture into heparinized tubes before and after 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

Table 1. Hematological characteristics of patients.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Bone marrow/Cell counts ($\times 10^4/µl$)</th>
<th>Blast (%)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>M</td>
<td>untreated</td>
<td>5.25</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>M</td>
<td>relapse</td>
<td>23.6</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>M</td>
<td>untreated</td>
<td>22.0</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>M</td>
<td>relapse</td>
<td>5.35</td>
<td>31</td>
</tr>
</tbody>
</table>

M: male.

protocol was approved by the Ethics Committee at the hospital, and informed consent was obtained from the subjects and/or their parents.

**Assay procedure.** The extraction procedure and chromatography method used were similar to those described by Puglisi C. V. and de Silva A. F. (5) and Bugge C. J. L. et al. (6). 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 for evaporation, the residue of which was dissolved in 100 μl of methanol and analyzed by high-performance liquid chromatography (HPLC). The HPLC system included a 655 Liquid Chromatogram pump (HITACHI, Tokyo) and a QC pack C-18 column (IRICA, Kyoto). The mobile phase for ATRA was 1,500 ml of acetonitrile, 1,000 ml of ammonium acetate buffer (v/v, 10%), and 100 ml of tetrahydrofuran at a flow rate of 1.5 ml/min. The mobile phase for 4-oxo retinoic acid was 1,000 ml of acetonitrile, 1,000 ml of ammonium acetate buffer (v/v, 5%), 100 ml of tetrahydrofuran, and 2 ml of acetic acid. The eluate was monitored at a wavelength of 340 nm using a spectrophotometric detector (IRICA, Kyoto). The recovery rate was 90–95% for both ATRA and 4-oxo ATRA, and the detection limit was 10 ng/ml. Authentic ATRA was purchased from Sigma Chemical Co. (St. Louis, MO, USA), while authentic 4-oxo ATRA was kindly supplied by Hoffmann-La Roche Co., Ltd. (Basel, Switzerland). The other chemicals used were obtained from Nacalai Tesque Co. (Kyoto, Japan).

![Graph](image)

**Fig. 1.** Plasma concentration of all-trans retinoic acid after administration of a single dose of all-trans retinoic acid (30 mg/m²). Patient Nos. 1–3 achieved complete remission, while patient No. 4 failed with all-trans retinoic acid therapy.

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Fig. 2. Plasma concentration of 4-oxo all-trans retinoic acid after administration of a single dose of all-trans retinoic acid (30mg/m²).

Results

Among the four APL patients given ATRA therapy, three achieved complete remission, while the other (patient No. 4) showed no objective response. Before ATRA therapy, ATRA and its derivatives were not detected in the plasma of any of the patients. After administration of the initial ATRA dose of 30mg/m², the peak plasma level was reached at 1–2h in all patients. In the three patients who achieved remission, the mean peak level was $471 \pm 303.2$ ng/ml (M ± SD), while the peak was only 20ng/ml in the patient who failed to achieve remission (Fig. 1). With respect to 4-oxo ATRA, in the patient without remission (No. 4) the peak level was 809ng/ml, while the other three patients with complete remission showed a peak level of $28.3 \pm 18.9$ ng/ml. In addition, the time to reach the peak 4-oxo ATRA level was 30 min in the patient without remission, while it was 120–240 min in the other three patients (Fig. 2).

Discussion

Although ATRA induces complete remission in a high proportion of APL patients, the duration of remission in patients with APL treated solely with ATRA has been relatively brief. Acquired resistance to ATRA may result from not only genetic or epigenetic causes, but also clinical pharmacological causes (4). Continuous treatment with ATRA induces a marked decrease in plasma ATRA level (7, 8). Muindi et al. (9) have shown that pharmacokinetic studies of APL patients who relapsed revealed a significant decrease in plasma ATRA levels compared to the first day of ATRA therapy. These facts suggests that low plasma ATRA levels may

be involved in the development of acquired resistance to ATRA.

ATRA is metabolized in vivo to produce 4-oxo ATRA, 4-oxo 13-cis retinoic acid \(^{(10)}\) and many other isomers, including 9-cis retinoic acid, 9,13-dicis retinoic acid and 13-cis retinoic acid \(^{(11)}\). Then, retinoids are excreted in glucuronised form in the bile. The decreased plasma level of ATRA indicates up-regulation of ATRA metabolism in patients receiving continuous therapy. It has been suggested that the increased metabolism is mediated by cytochrome P-450 oxidation \(^{(12)}\), increased expression of cellular retinoic acid-binding proteins \(^{(13)}\), and increased glucuronidation for excretion in the bile, with down-regulation of gastrointestinal absorption also being postulated \(^{(14)}\). The accelerated metabolism of ATRA may explain the low plasma ATRA levels and the failure to achieve effective ATRA intracellular levels.

In our pediatric APL patients who received ATRA therapy, a substantial level of 4-oxo ATRA was detected in the plasma. It is noteworthy that in the patient without remission, a very low plasma ATRA level was documented in association with a far higher 4-oxo ATRA level than in the patients with remission. This indicates that the lack of remission may have been due to failure to achieve an effective plasma level in this patient, because of enhanced metabolism of ATRA as evidenced by the extremely elevated 4-oxo ATRA level. In this patient, the initiation of ATRA therapy may accelerate the ATRA metabolism of the second therapy.

Many factors may influence the pharmacokinetic behavior of oral administration of ATRA, including gastrointestinal absorption and ATRA metabolism \(^{(15)}\). Further studies are needed to clarify the metabolism of ATRA. However, we conclude that it is useful to monitor the plasma concentration of ATRA and its metabolites in order to achieve an appropriate treatment regimen.

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REFERENCES


