Effectiveness and Pharmacokinetics of Low-Dose All-*trans* Retinoic Acid (25 mg/m^2) in Acute Promyelocytic Leukemia

By Sylvie Castaigne, Philippe Lefebvre, Christine Chomienne, Etienne Suc, Francoise Rigal-Huguet, Claude Gardin, Alain Delmer, Eric Archimbaud, Hervé Tilly, Maud Janvier, Françoise Isnard, Philippe Travade, Luc Montfort, A. Delannoy, Marie Josée Rapp, Bernard Christian, Marion Montastruc, Hans Weh, Pierre Fenaux, Hervé Dombret, Bernard Gourmel, and Laurent Degos

It has been shown that all-*trans* retinoic acid (ATRA) at doses of 45 to 100 mg/m^2/d induces complete remission (CR) of acute promyelocytic leukemia (APL) by a differentiation process. To date, ATRA dose-ranging studies have not yet been evaluated. Thus, we initiated in May 1990 a multicenter study with ATRA at a lower dose of 25 mg/m^2/d until CR. Thirty patients with APL were treated with ATRA, of whom 12 were previously untreated, 14 were in first relapse, and 4 had failed after conventional first induction chemotherapy. Twenty-four of 30 achieved CR, 3 failed, and 3 died before day 30. Median time to CR was 45 days. Hyperleucocytosis (14 to 43 × 10^9 white blood cells per liter) was observed in 9 patients between days 10 and 23. Clinical complications that may have been related to the retinoic acid syndrome were observed in 8 patients, of whom 3 died. Pharmacokinetics studies were performed in 5 patients. Peak plasma concentrations and mean area under the concentration-time curve were not lower than previous levels obtained under the 45 mg/m^2 dose. Overall, our study shows that there is no difference in terms of therapeutic efficacy, triggering of hyperleukocytosis, or retinoic acid syndrome and pharmacokinetic results with ATRA at 25 or 45 mg/m^2/d.

ACUTE PROMYELOCYTIC leukemia (APL) is a particular form of acute myeloid leukemia with a distinct cytologic morphology (M3 and M3 variant in the French-American-British [FAB] classification),[1,2] specific chromosomal translocation t(15;17),[3] and high incidence of coagulation disorders.[4] It has been shown that the t(15;17) disrupts the retinoic acid receptor α gene (RARα) and fuses it to a new gene called PML.[5] In vitro studies have shown differentiation of fresh APL leukemia cells in the presence of all-*trans* retinoic acid (ATRA).[6] The first in vivo study was performed in China with ATRA at doses ranging from 30 to 100 mg/m^2/d.[7] The dose of ATRA administered until complete remission (CR) by us and others was 45 mg/m^2/d.[8-10] These studies have shown high efficacy of ATRA with a response obtained in nearly all newly diagnosed or first-relapsing patients. These studies have pointed out particular clinical and biologic side effects supervening during ATRA treatment. First, retinoic acid syndrome with fever, pulmonary infiltrates, pericardial or pleural effusions, and renal insufficiency, most often associated with hyperleukocytosis, can be observed during the first 3 weeks of therapy.[11,12] Retinoic acid syndrome and hyperleukocytosis may recede spontaneously, but may lead to life-threatening complications. Second, failure of remission maintenance therapy with ATRA alone is frequently concomitant to a progressive reduction in plasma drug concentration[13] and an induction of cytoplasmic retinoic acid binding proteins in leukemic cells.[14] Because ATRA dose-ranging studies have not yet been evaluated as well as to reduce these two major limitations of ATRA treatment, we began in May 1990 an open multicentric study in APL with ATRA at a lower dose, ie, 25 mg/m^2/d.

PATIENTS AND METHODS

From May 1990 to January 1992, 30 patients with an M3 leukemia from 15 different institutions in France, Belgium, and Germany were treated in their hospital of origin. Karyotypic analysis was available in 26 patients and showed the specific t(15;17) in 25. ATRA at a dose of 25 mg/m^2/d was administered in two oral doses until CR. Blood counts and coagulation tests were performed at least thrice weekly. Bone marrow examination was performed each month and/or at the time of response evaluation. Complete response was defined by the disappearance of APL blasts in the bone marrow and the normalization of blood counts for at least 2 months. Supportive care, prevention or treatment of the retinoic acid syndrome, and maintenance therapy were left to each center's decision. Informed consent was obtained from all patients.

Patients. Thirty patients (13 male and 17 female) with a mean age of 56 years (range, 10 to 81) were treated. Twelve patients were treated for a newly diagnosed disease because of contraindication to conventional chemotherapy. There were 9 elderly patients (66 to 81 years), 2 obese patients with cardiac disease, and 1 human immunodeficiency virus (HIV)-positive patient. Fourteen patients were treated in first relapse after conventional chemotherapy. Four patients were treated after failure of conventional chemotherapy in-

From the Department of Hematology, Hopital Saint Louis, Paris; the Department of Hematology, Hopital Purpan, Toulouse; the Department of Hematology, Hopital Beaujor, Paris; the Department of Hematology, Hopital Hotel-Dieu, Paris; the Department of Hematology, Hopital Ed. Herriot, Lyon; the Department of Hematology, Centre Anticancéreux Becquerel, Rouen; the Department of Hematology, Hopital René Huguenin, St Cloud; the Department of Hematology, Hopital St Antoine, Paris; the Department of Hematology, Centre Jean Perrin, Clermont Ferrand; the Department of Hematology, Hopital Hotel-Dieu, Nantes; the Department of Hematology, Hopital de Metz; the Department of Hematology, Hopital du Haut Lévêque, Pessac; the Department of Hematology, Hopital Cl. Huriez, Lille; the Department of Hematology, Clinique St Luc, Bouge, Belgique; the Department of Hematology, Hopital de Jolimont, Belgium; the Department of Hematology, University of Hambourg, Germany; the Department of Biochemistry, Paris; and the Laboratory of Analytic Chemistry, Faculté de Pharmacie, Paris, France.

Submitted May 24, 1993; accepted August 12, 1993.

Address reprint requests to Sylvie Castaigne, MD, Department of Hematology, Hopital Saint Louis, 1, avenue Claude Vellefaux, 75010 Paris, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/93/8212-0015$3.00/0

duction. No patient presented with hyperleukocytosis before the onset of ATRA (white blood cell count [WBC], 0.2 to 7.6 $\times$ 10^9/L; median, 1.6 $\times$ 10^9/L). Coagulopathy (fibrinogen <200 mg/dL and/or elevation of fibrinogen degradation products or D dimers) was present in 8 patients.

Pharmacokinetics. Plasma pharmacokinetics were performed in 5 patients after the administration of a single oral dose of ATRA using a new method. Briefly, blood samples were collected in aluminum foil wrapped heparized tubes before ingestion, and again at 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, and 24 hours, thereafter. After a solid phase extraction, the samples were analyzed by high performance liquid chromatography (HPLC) on a silica column in isocratic mode with UV detection (360 nm). In this method, the internal standard was the RO-10.1670 (isotretinoin). All samples were rigorously taken, stored, and manipulated in the dark or under subdued light. Model independent pharmacokinetic parameters were calculated for each patient. Elimination half-lives (t1/2B) were estimated by linear regression after log transformation of the concentrations. The area under the concentration-time curve (AUC) was calculated by the trapezoidal rule.

RESULTS

Induction therapy. Induction therapy results are shown in Table 1. Among the 12 patients treated for a newly diagnosed disease, 10 reached CR between days 30 and 90 (3 on day 30, 2 on day 45, 3 on day 60, and 2 on day 90). Two patients died: one on day 6 from cerebral hemorrhage (WBC, 1.6 $\times$ 10^9/L at diagnosis, and 9 $\times$ 10^9/L at the time of death), and one on day 17 with acute respiratory distress syndrome (ARDS; WBC, 0.9 $\times$ 10^9/L at diagnosis and 14 $\times$ 10^9/L on day 13).

Among the 14 patients treated in first relapse, 10 reached CR (4 on day 30, 2 on day 45, 2 on day 60, and 2 on day 90). One patient had a transient partial response. t(15;17) was present at diagnosis in this patient. One patient died from ARDS on day 23 (WBC, 7 $\times$ 10^9/L at the beginning of ATRA therapy and 34 $\times$ 10^9/L on day 10). Two patients were considered as failures to respond. One patient failed to respond after 45 days of ATRA therapy and despite ATRA dose escalation to 45 mg/m^2 on day 30. The other patient presented with renal and pericardial effusion on day 26, leading to discontinuation of ATRA therapy and high-dose dexamethazone therapy with clinical improvement. ATRA therapy was therefore started again without response and the patient died on day 50. These two patients had t(15;17) on karyotypic examination before treatment.

Table 1. Results of Induction With ATRA

	CR	PR	Death	Failure
Newly diagnosed (N = 12)	10		2	
First relapse (N = 14)	10	1	2	1
Induction failure (N = 4)	4	0	0	0

Date and cause of death: day 6, intracranial hemorrhage; day 17, cardiorespiratory failure (ATRA syndrome; WBC, 14 $\times$ 10^9/L on day 13); day 23, ARDS (ATRA syndrome; WBC, 34 $\times$ 10^9/L on day 10); day 50, ATRA syndrome and resistance.

Abbreviation: PR, partial remission.

The 4 patients treated with ATRA therapy after failure of conventional chemotherapy entered CR on days 15 to 30 (1 on day 15, 3 on day 30).

Maintenance treatment and response durations. Results are indicated in Table 2. One elderly patient (80 years old) did not receive any maintenance therapy because of psychologic reasons (severe depression) and died 2 months later from a cause unrelated to her hematologic disease. The result of maintenance treatment was not known for 1 patient treated with ATRA after failure of induction.

Five patients received a consolidation course with daunorubicin (45 mg/m^2/d) for 3 days and Ara-C (200 mg/m^2/d) for 7 days, followed by maintenance therapy with 6-mercaptopurine and methotrexate or low-dose Ara-C. These patients are in continuous CR on month 6 to 14. Eleven patients received three monthly mild consolidation courses as outpatients with anthracyclins or amsacrine on day 1 and cytosine arabinoside by subcutaneous injections for 5 days. Eight of these patients have relapsed (median duration of CR, 10 months). Three patients received an autologous bone marrow transplant: 1 died of the procedure, 1 has relapsed on month 19, and 1 is alive in CR after 14 months. One patient received low-dose Ara-C and continues to be in CR on month 8. One patient continued ATRA alone and relapsed on month 3. One patient was treated with consolidation courses as an outpatient and then received an allogenic bone marrow transplantation with an unrelated donor and is alive in CR 6 months after.

One newly diagnosed patient who achieved CR with ATRA therapy and received mild consolidation courses as an outpatient and then maintenance treatment with 6-mercatopurine and methotrexate relapsed 2 years later. He was treated again with ATRA therapy and remission was easily obtained.

Clinical and biologic side effects. Clinical complications occurred in 11 patients: cerebral hemorrhage on day 6, ARDS in 2 patients, isolated fever of unknown origin in 1 patient or associated with pleural effusions in 2 patients, pulmonary infiltrates in 1, cutaneous erythema in 1, arthralgias in 1, and fever concomitant to clinical evidence of infections in 2 patients. Four patients died: 1 of cerebral hemorrhage on day 6, 2 with ARDS on days 17 and 23, and 1 with ATRA resistance on day 50. Overall, 8 patients experienced some of the clinical symptoms that may be related to the retinoic acid syndrome. Complications related to ATRA therapy may have been the cause of death in 3 patients.

Hyperleukocytosis (WBC, > 10 $\times$ 10^9/L) was observed in 9 patients who achieved a maximal WBC count (14 to 43 $\times$ 10^9/L) between days 10 and 23. Hyperleukocytosis was associated with clinical complications in 6 of 9. In 5 patients, hyperleukocytosis was not treated: 3 entered CR (maximum WBC count, 25 $\times$ 10^9/L on day 23, 15 $\times$ 10^9/L on day 20, 32 $\times$ 10^9/L on day 10) and 2 died of ARDS (maximum WBC count, 14 $\times$ 10^9/L on day 13; 34 $\times$ 10^9/L on day 10). The other 4 patients received either chemotherapy or dexamethazone. Two elderly patients (aged 81 and 76 years) were treated with chemotherapy. The first patient received 6-mercaptopurine at 150 mg/d starting on day 20 (WBC

Table 2. Duration of CR

	Consolidation "3 + 7"	Consolidation as Outpatients "1 + 5"	ABMT	Low-Dose ARA-C	ATRA
Newly diagnosed patients (9)	14+, 9+, 6+	12+, 22, 20, 10, 4		8+	
First relapse (9)	9+, 6+	26+, 20, 8, 4	14+, 3		3
Induction failure (3)		4+, 7	19		

One patient not included in the table received an ABMT.
Abbreviation: ABMT, allogenic bone marrow transplantation.

count, 32×10^9/L); the second one received VP16 at 175 mg for 1 day and cytosine arabinoside at 180 mg/d for 5 days, starting on day 23 (WBC count, 16×10^9/L). Both patients entered CR. In 2 patients (maximum WBC count, 43×10^9/L on day 10 and 20×10^9/L on day 15), ATRA therapy was stopped for 1 to 2 weeks and dexamethazone was introduced; both reached CR.

Extra hematologic side effects such as headache and dryness of mucosae were considered negligible.

Pharmacokinetics. ATRA plasma pharmacokinetic studies were performed in 5 patients. The time to peak plasma concentrations was 60 to 240 minutes (mean, 132 minutes). The peak plasma concentration varied from 0.22 to 4.34 μg/mL (mean, 1.63 μg/mL), and mean AUC was 733 ngH/mL.

DISCUSSION

The data presented here show that ATRA at a dose of 25 mg/m^2/d is quite effective in inducing CR in M3 patients as 24 of 30 patients reached CR.

Among the 6 patients who did not reach CR, 3 died before evaluation: 1 newly diagnosed patient died early from cerebral hemorrhage, 2 patients (one newly-diagnosed and one first relapsing) died at week 3 of therapy of an ARDS concomitant with hyperleucocytosis. One patient treated in first relapse had a transient response. Two patients treated both in first relapse may be considered refractory to ATRA. However, these 2 patients had the specific t15;17 on karyotypic analysis. Our results are not different from those observed in previous studies with ATRA at a dose of 45 mg/m^2/d.[7-10] In our first study, we observed among 22 patients 3 early deaths, 4 transient responses, 1 resistance, and 14 CR.[8] Median time to CR (45 days) is also within the range observed with a higher dose.

Hyperleukocytosis (WBC $>10 \times 10^9$/L) was observed in one-third of the patients. Again, this frequency is not different from previous studies using ATRA at a dose of 45 mg/m^2/d. In our first study among 15 leukopenic patients at the beginning of ATRA therapy, 4 presented an hyperleukocytosis greater than 10×10^9/L. Frankel et al[12] reported 14 of 35 patients with hyperleukocytosis greater than 20×10^9/L.

Fourteen patients experienced clinical or biologic symptoms under ATRA. In 8 of them, clinical symptoms may be related to the retinoic acid syndrome[12] and in 3 patients ATRA side effects may have been the cause of death. On the contrary, ATRA was extremely well tolerated without any biologic or clinical side effects in 16 patients (4 of 12 in first induction, 9 of 14 relapsing, and 3 of 4 after induction chemotherapy failure). As in our previous study, we found a relationship between hyperleukocytosis and the occurrence of clinical side effects (Table 3). But, as pointed out in the study of Frankel et al,[12] this relationship is not absolute and some patients may experience well-tolerated hyperleukocytosis, whereas severe retinoic acid syndrome may occur concomitantly with a moderate increase in leukocytes. However, once established, retinoic acid syndrome, especially with pulmonary complications, is difficult to manage. For this reason, in a European multicentric prospective trial using ATRA as treatment for newly diagnosed patients, it was decided to treat patients who developed hyperleukocytosis with full-dose chemotherapy.[15] In the study presented here, which includes patients with contraindication to conventional chemotherapy, management of hyperleukocytosis and/or retinoic acid syndrome was decided locally. Because of the small number of patients studied, it is difficult to conclude in favor of either chemotherapy or dexamethazone treatment. Moreover, as previously reported, in 3 cases, hyperleukocytosis without clinical complications did not require treatment and receded spontaneously after day 21.

Thus, in our experience, there is no difference in terms of therapeutic efficacy, triggering of hyperleukocytosis, or retinoic acid syndrome with ATRA at 25 or 45 mg/m^2/d. This is not surprising when we refer to the pharmacokinetics results. Peak plasma concentration varied from 0.22 to 4.34 μg/mL. In our previous study with 45 mg/m^2, peak plasma concentrations were in the same range (0.03 to 2.5 μg/mL; mean, 0.4 μg/mL), and the mean AUC level was not different (AUC 45 mg/m^2, 630 ngH/mL; AUC 25 mg/m^2, 733 ngH/mL),[16] similar to another pharmacokinetic study of ATRA 45 mg/m^2 (AUC on day 1, 682 ngH/mL).[17] Despite the wide interpatient variations in ATRA plasma level obtained with the different administered doses, it is clear that the levels obtained with 25 mg/m^2 are not significantly lower than those with 45 mg/m^2.

Besides the risk of hyperleukocytosis, induction of resis-

Table 3. Correlation Between Hyperleukocytosis and Clinical Complications

	Clinical Complications	
	+	−
Hyperleukocytosis		
+	6	3
−	5	16

$P < .05$

tance to ATRA therapy is another main side effect of this treatment. Mechanisms of resistance are not clearly understood. It has been shown that chronic oral administration of ATRA resulted in a progressive decrease in peak plasma concentrations and in AUC values, with an increase of urinary elimination of its metabolite suggesting an increased catabolism of the drug.[17] Increased catabolism may be due to induction of cytochrome P450-like enzyme systems and/or to an increase of cytoplasmic retinoic acid binding proteins in normal tissues that could clear the drug from plasma.[14] In this study, only 1 patient, who achieved CR with ATRA as a first induction, was treated again with ATRA when relapse occurred 2 years later. A second remission was easily obtained with ATRA. This single observation does not permit us to conclude that absence of acquired resistance was caused by reduced ATRA doses used during the first treatment. An alternative explanation may also be the long withdrawal (2 years) from ATRA between two treatments. If the acquired resistance is generally reported, one study mentioned that a second reinduction remission with ATRA was possible in patients treated for a short period with this drug.[10]

ATRA is a novel and very promising therapy in APL. Recent studies have shown that ATRA followed by or combined with conventional chemotherapy may be more beneficial for patients than chemotherapy alone.[15] However, a subset of APL patients, elderly or relapsing, are not candidates for conventional chemotherapy. Further studies about the appropriate doses or length of ATRA therapy are needed to optimize this therapy, both as an induction and maintenance treatment, in de novo and relapsing APL patients. We recently initiated a study with ATRA 15 mg/m^2/d until CR in elderly or first relapsing patients. Preliminary results show an equal efficacy and tolerance.

REFERENCES

1. Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C: Proposals for the classification of the acute leukemias. Br J Haematol 33:451, 1976

2. Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C: A variant form of hypergranular promyelocytic leukemia (M3). Br J Haematol 44:169, 1980

3. Larson RA, Kondo K, Vardiman JW, Butler AE, Golomb HM, Rowley JD: Evidence for a 15;17 translocation in every patient with acute promyelocytic leukemia. Am J Med 76:827, 1984

4. Tallman MS, Kwaan HC: Reassessing the hemostatic disorder associated with acute promyelocytic leukemia. Blood 79:543, 1992

5. De Thé H, Chomienne C, Lanotte M, Degos L, Dejean A: The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor alpha gene to a novel transcribed locus. Nature 347:558, 1990

6. Chomienne C, Ballerini P, Balitrand N, Daniel MT, Fenaux P, Castaigne S, Degos L: All-*trans* retinoic acid in acute promyelocytic leukemia. II: In vitro studies: structure-function relationship. Blood 76:1710, 1990

7. Huang M-E, Ye Y-C, Chen S-R, Chai J-R, Lu J-X, Zhoa L, Gu L-J, Wang Z-Y: Use of all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72:567, 1988

8. Castaigne S, Chomienne C, Daniel MT, Ballerini P, Berger R, Fenaux P, Degos L: All-*trans* retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood 76:1704, 1990

9. Warrell RP Jr, Frankel SR, Miller WH, Scheinberg DA, Itri LM, Hittelman WN, Vyas R, Andreeff M, Tafuri A, Jakubowski A, Gabrilove J, Gordon MS, Dmitrovsky E: Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans* retinoic acid). N Engl J Med 324:1385, 1991

10. Chen Z-X, Xue Y-Q, Zhang R, Tao R-F, Xia X-M, Li C, Wang W, Zu W-Y, Yao X-Z, Ling B-J: A clinical and experimental study on all-*trans* retinoic acid treated acute promyelocytic leukemia patients. Blood 78:1413, 1991

11. Castaigne S, Chomienne C, Fenaux P, Daniel MT, Degos L: Hyperleucocytosis during all-*trans* retinoic acid for acute promyelocytic leukemia. Blood 76:260a, 1990 (abstr, suppl 1)

12. Frankel SR, Eardley A, Lauwers G, Weiss M, Warrell RP Jr: The "retinoic acid syndrome" in acute promyelocytic leukemia. Ann Intern Med 117:292, 1992

13. Muindi J, Frankel SR, Miller WH, Jakubowski A, Scheinberg DA, Young CW, Dmitrovsky E, Warrell RP Jr: Continuous treatment with all-*trans* retinoic acid causes a progressive reduction in plasma drug concentrations: Implications for relapse and retinoid "resistance" in patients with acute promyelocytic leukemia. Blood 79:299, 1992

14. Cornic M, Delva L, Guidez F, Balitrand N, Degos L, Chomienne C: Induction of retinoic acid binding protein in normal and malignant human myeloid cells by retinoic acid in AML3 patients. Cancer Res 52:3329, 1992

15. Fenaux P, Castaigne S, Dombret H, Archimbaud E, Duarte M, Morel P, Lamy T, Tilly H, Guerci A, Maloisel P, Bordessoule D, Sadoun A, Tiberghien P, Fegueux N, Daniel MT, Chomienne C, Degos L: All-*trans* retinoic acid followed by intensive chemotherapy gives a high complete remission rate and may prolong remissions in newly diagnosed acute promyelocytic leukemia: A pilot study on 26 cases. Blood 80:2176, 1992

16. Lefebvre P, Thomas G, Gourmel B, Agadir A, Castaigne S, Dreux C, Degos L, Chomienne C: Pharmacokinetics of oral all-trans retinoic acid in patients with acute promyelocytic leukemia. Leukemia 5:1054, 1991

17. Muindi JR, Frankel SR, Huselton C, DeGrazia F, Garland WA, Young CW, Warrel RP Jr: Clinical pharmacology of oral all-trans retinoic acid in patients with acute promyelocytic leukemia. Cancer Res 52:2138, 1992