Combination Therapy with Granulocyte Colony-Stimulating Factor, *All-Trans* Retinoic Acid, and Low-Dose Cytotoxic Drugs for Acute Myelogenous Leukemia

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A 67-year-old man presented with acute myelogenous leukemia (M2). Peripheral blood examination revealed a leukocyte count of 1,700/ μ l with 1% myeloblasts, and bone marrow aspiration showed 42.6% myeloblasts with Auer bodies. Culture of his marrow cells at diagnosis showed that granulocyte colony-stimulating factor (G-CSF) promoted cell proliferation, while *all-trans* retinoic acid (ATRA) inhibited the proliferative effect of G-CSF and induced differentiation. Combination therapy with G-CSF, ATRA, and low-dose cytotoxic drugs achieved complete remission without severe marrow suppression. (Internal Medicine 34: 1186–1189, 1995)

Key words: differentiation, low-dose cytosine arabinoside therapy, cytarabine ocfosfate

Introduction

Low-dose cytosine arabinoside (araC) is widely used for myelodysplastic syndrome and in elderly patients with acute myelogenous leukemia (AML) (1, 2). It was originally reported as a differentiation therapy but it frequently induces myelosuppression due to its cytotoxic effects (3). *All-trans* retinoic acid (ATRA) appears to be the most effective agent for promoting the differentiation of acute promyelocytic leukemia, a subtype of AML (4). We present a case of AML (M2) in which treatment with a combination of ATRA, granulocyte-colony stimulating factor (G-CSF), cytarabine ocfosfate (a prodrug of araC) (5, 6), and low-dose cyclophosphamide achieved complete remission without severe marrow suppression.

Case Report

A 67-year-old man was admitted to our hospital with fever on March 22, 1993. Peripheral blood examination revealed that the leukocyte count was 1,700/ μ l with 1% atypical myeloblasts and 56.5% mature granulocytes. In addition, the hemoglobin was 8.6 g/dl, the platelet count was 15.4×10^4 / μ l, the level of fibrin/fibrinogen degradation products (FDP) was 84 μ g/dl, the thrombin-antithrombin complex level was 39.0 ng/ml, and the α 2 plasmin inhibitor-plasmin complex level was 4.8 μ g/ml. Bone marrow aspiration showed slightly hypocellular marrow

and 42.6% myeloblasts with Auer bodies. Chromosomal analysis of the marrow cells disclosed a karyotype of 56, XY, +X, +X, +1, +2, del(5)(q13,q33), +8, +10, +11, +14, +20, +22. These findings led to the diagnosis of M2 AML according to the FAB classification (7) associated with disseminated intravascular coagulation (DIC). [3 H]thymidine incorporation assay of cultured marrow cells obtained at diagnosis showed that G-CSF promoted cell growth and that ATRA inhibited this effect of G-CSF (Fig. 1). Morphological examination of cells in liquid culture revealed that ATRA induced modest differentiation and that G-CSF did not block this differentiating effect of ATRA (Table 1). He had suffered from pneumoconiosis since 1981, and blood gas analysis revealed a pH of 7.41, a PaO₂ of 61.5 mmHg, a PaCO₂ of 60.4 mmHg, and an O₂ saturation of 91.4%.

Because of his chronic respiratory failure and the in vitro data, we administered ATRA (Tretinoin[®], provided by NIH, Bethesda, MD, USA; 45 mg/m² = 70 mg daily) and cytarabine ocfosfate (SPAC; a prodrug of cytosine arabinoside; 50 mg daily/ Starasid[®], Nippon Kayaku, Tokyo, Japan) together with heparin after obtaining informed consent. Within 2 weeks, his DIC was well controlled by heparin therapy and FDP became undetectable. After administration of ATRA and SPAC for 36 days, marrow leukemic blasts decreased to 19.2%, but the peripheral blood leukocyte count decreased to 1,500/ μ l with a decline of mature granulocytes (15%). The results of the above in vitro studies plus the onset of sepsis and neutropenia prompted

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Figure 1. Effect of all-*trans* retinoic acid and granulocyte colony-stimulating factor on the growth of bone marrow cells cultured from the patient at the time of initial diagnosis. White columns and black columns indicate [³H]thymidine incorporation (cpm) in the presence of the indicated concentrations of granulocyte colony-stimulating factor with and without 10⁻⁶ M retinoic acid, respectively. Bars indicate the SD of triplicate experiments. The assay was done according to the method of Tsuchiya et al (22).

us to start subcutaneous administration of G-CSF (Filgrastim, Kirin, Tokyo, Japan) at a daily dose of 75 µg in combination with ATRA and SPAC. Within 4 days, the peripheral leukocyte count increased to 2,700/µl with an increase of mature granulocytes (44%). After administration of G-CSF, ATRA, and SPAC for 36 days, leukemic blast cells decreased to 4.6%

Table 1. Effect of all-*trans* retinoic acid and granulocyte colony-stimulating factor on differentiation of the patient's bone marrow cells in liquid culture. The assay was done as described by Motoji et al (21). Briefly, cells were collected by sternal aspiration at the time of initial diagnosis and subjected to liquid culture. After 4 days of incubation with the indicated concentrations of granulocyte colony-stimulating factor and retinoic acid, the cells were examined.

G-CSF (ng/ml)	ATRA (M)	Immature blasts (%)	Mature granulocytes (%)
0	0	73.5±2.1	24.7±2.1
0	10 ⁻⁶	67.3±3.2	32.7±3.2
10	0	70.3±4.0	29.7±4.0
10	10 ⁻⁶	67.3±2.5	32.7±2.5

Figure 2. Clinical course of the patient. CPA: cyclophosphamide, NCC: nucleated cell counts.

in the bone marrow. Hemoglobin concentration also gradually decreased to 7.5 g/dl. However, following administration of a higher dose of SPAC (200 mg daily) with G-CSF and ATRA for 18 days, marrow blasts increased to 10%, therefore we added oral cyclophosphamide at 50 mg daily. Following administration of cyclophosphamide in combination with ATRA, G-CSF, and SPAC for 14 days, marrow blasts decreased to 1%. On day 122 of treatment, peripheral blood and bone marrow examination showed complete remission.

Discussion

In the present case, combined administration of ATRA and SPAC (50 mg/day) produced a favorable response. SPAC (1- β -D-arabinofuranosylcytosine 5'-stearylphosphate) is a prodrug of cytosine arabinoside, and its oral administration at a daily dose of 100–300 mg is reported to give an equivalent plasma araC concentration to that achieved with low-dose araC (10 mg/m² twice daily) (5, 6), which is a cytotoxic chemotherapy but is also known as a differentiation therapy for AML (2). Since low-dose araC has a cytotoxic effect and sometimes induces severe myelosuppression (3), very-low-dose araC therapy (3 mg/m² twice daily) has also been developed (8, 9). It has been shown to be as effective as a low-dose regimen (10), despite a peak plasma araC concentration at least 10-fold lower than in the other regimen (11). Thus, the administration of SPAC at 50 mg/day may correspond to very-low-dose araC therapy. ATRA is the most effective agent for producing the differentiation of acute promyelocytic leukemia (4). Brief exposure to ATRA is reported to increase the in vitro araC sensitivity of cells from two established AML cell lines (12), and therapy with low-dose araC plus ATRA is reported to induce severe myelosuppression (13), as also occurred in the present case.

G-CSF usually promotes the growth of AML cells in vitro (14). The combination of G-CSF or GM-CSF with chemotherapy has been reported to induce severe myelosuppression or leukemic regrowth (15–18). On the other hand, it was reported that G-CSF enhances the in vitro differentiation of AML cells in the presence of ATRA (19) and that ATRA inhibits the G-CSF-induced proliferation of hemopoietic progenitor cells (20). In the present case, ATRA inhibited the promotion of the growth of immature blasts by G-CSF, although this cytokine did not inhibit the in vitro slight differentiation effect of ATRA. Moreover, the addition of G-CSF to the patient's therapy ameliorated the marrow suppression induced by the combination of ATRA and SPAC without obvious leukemic regrowth, and helped to achieve a good response. Following the subsequent addition of low-dose cyclophosphamide, complete remission was achieved without severe myelosuppression. These findings suggest the possibility that in the present case the combined administration of G-CSF and ATRA enhanced the differentiation effect of SPAC which was equivalent to very low-dose araC therapy (2) or the alternative possibility that ATRA played a role in the augmentation of antileukemic activity by SPAC and inhibition of leukemic regrowth promoted by G-CSF which stimulated granulopoiesis. Further

investigation is needed to clarify the most suitable schedule for the combined administration of ATRA, low dose araC, G-CSF, and other cytotoxic drugs in AML.

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