Hematological and Biochemical Action of Tiazofurin (NSC 286193) in a Case of Refractory Acute Myeloid Leukemia

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ABSTRACT

A patient with refractory acute myeloid leukemia was treated with tiazofurin, an agent that causes inhibition of tumor cell proliferation by depressing glutamic pyruvate transaminase concentrations in the malignant cells. The initial dose of 1100 mg/m² was ineffective clinically and biochemically. Dose escalations to 1650, 2200, and finally 3300 mg/m² resulted in a marked decrease in the absolute number of blasts without causing bone marrow hypoplasia or marked neutropenia. The decrease in the peripheral blast cell count was observed subsequent to a decline in glutamic pyruvate transaminase concentrations in the leukemic cells to <30% of the pretreatment value. Consecutive bone marrow examinations showed a remarkable shift from myeloblasts to more mature myeloid elements, suggesting an in vitro differentiative action of tiazofurin. Although a total dose of 23,650 mg/m² was administered over a 13-day period, only very mild side effects were noted. The absence of complications included by others in Phase I trials with tiazofurin may be related to our slow administration of the drug by pump over a 1-h period in this trial. Tiazofurin appears to be a promising agent in the treatment of leukemia because of its selective action on leukemic cell types and the availability of a rapid in vitro method capable of predicting sensitivity of leukemic cells to the agent and monitoring its activity during treatment by measuring thiazole-4-carboxamide adenine dinucleotide and glutamic pyruvate transaminase concentrations. These observations are being tested in a larger group of leukemic patients.

INTRODUCTION

The combination of cytarabine with an anthracycline is an effective therapy for AML; only a small percentage of patients, perhaps less than 10%, are truly resistant to these agents (1). The long held hope, however, that antileukemic drugs in AML would have a selective effect on leukemic cells while sparing the normal hematopoietic and other rapidly dividing cells has not been realized. Essentially, very hypoplastic marrows need to be produced before a complete remission can be attained in AML. Current chemotherapeutic regimens used in AML always cause significant toxicity, but do not always produce antileukemic effects, especially in relapsed AML. It is difficult to clinically evaluate the therapeutic effects of a regimen before the entire course of therapy is administered. The availability of in vitro methods capable of determining the sensitivity of leukemic cells to specific chemotherapeutic agents would greatly facilitate the treatment of this disease by providing means for rationally selecting induction regimens appropriate for individual patients. To date, the measurement of the killing effect on leukemic cells which clone in vitro appears to provide the most clinical information. The cloning methods, however, are tedious to carry out, they require 7 to 10 days to provide information and, therefore, are of little practical use (2).

Tiazofurin (NSC 286193), a C-nucleoside, is an analogue of the antiviral agent ribavirin. While tiazofurin has little antiviral activity, it exhibits potent antitumor activity against murine tumors (3-6). Tiazofurin is metabolized to thiazole-4-carboxamide adenine dinucleotide, TAD, a potent inhibitor of IMP dehydrogenase activity (7). This causes depression of GTP and dGTP pools leading to inhibition of tumor cell proliferation (6, 8). Jayaram et al. (9) have recently provided evidence that human leukemic cells are selectively sensitive to tiazofurin. When labeled tiazofurin was incubated with leukocytes obtained from healthy volunteers or from leukemic patients, the leukemic cells produced over 20-fold higher concentrations of TAD than the normal leukocytes. Incubation with tiazofurin in leukemic leukocytes decreased the GTP pool (to 48 to 79%), whereas there was no change in the normal leukocytes. Thus, incubation of leukemic cells with tiazofurin and measuring TAD formation and GTP concentration after 2 h should constitute a rapid predictive test to determine whether the leukemic cells are sensitive or resistant to tiazofurin treatment (9).

We report here the results of treatment with tiazofurin in a patient with refractory AML. There was a remarkable reduction in the GTP concentrations in the blast cells and a subsequent decline in the blast cell number in the peripheral blood and the bone marrow. There was a close correlation between the depression of GTP concentrations in the leukemic cells and the clinical effect. Consecutive bone marrow samples provided evidence for a differentiative action of tiazofurin. The patient did not experience important side effects during the whole course of the treatment.

MATERIALS AND METHODS

Patient. The patient was a 48-yr-old white male who was diagnosed with AML in November 1985 in an outside hospital. He was treated with a regimen consisting of daunorubicin (60 mg/m²) for 3 days and cytarabine and 6-thioguanine (100 mg/m²) every 12 h for 7 days. No remission was attained, and he was subsequently treated with high-dose cytarabine (3 g/m²) every 12 h for 4 days and daunorubicin (50 mg/m²) on the fifth day. The patient entered complete remission and received two consolidation courses with the same regimen at the same dose. He relapsed in October 1986 and was treated with amsacrine (200 mg/m²) for 5 days. Because no remission was attained, he was treated with etoposide (150 mg/m²) also for 5 days, again without success. He then received two courses of high-dose cytarabine (3 g/m²) every 12 h for 4 days followed by asparaginase (10,000 units/m²). This resulted in a complete remission, but therapy was complicated by transient cerebellar toxicity. He relapsed again in March 1987 and was referred to our hospital for further treatment. On admission, the patient was in good clinical condition with a Karnovsky score of 90%. Physical examination was normal. Laboratory examination showed the following data: hemoglobin (9.3 g/dl); hematocrit (0.27); platelets (36 x 10¹²/liter); WBC (25.5 x 10¹²/liter with a differential showing 60% blasts, 3% myelocytes, 20% segmented neutrophils, 2% eosinophils, 13% lymphocytes, and...
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2% monocytes). Electrolytes and renal function were normal. Liver function tests were slightly disturbed: alkaline phosphatase [133 units/liter (normal, 25 to 125)], SGPT [80 units/liter (normal, 0 to 35)], and \( \gamma \)-glutamyltransferase [90 units/liter (normal, 5 to 55)]; bilirubin and SGOT were normal. Bone marrow examination showed a hypercellular specimen with a marked decrease in megakaryocytes and erythroblasts; the myeloid series was hyperplastic and mainly represented by blast cells: 74%. Cytogenetic analysis revealed a normal karyotype.

Biochemical Assays. Preparation of bone marrow and peripheral leukocytes, measurement of the conversion of labeled tiazofurin to TAD, and determination of the concentrations of GTP were carried out as reported (9). In short, leukocytes were separated on a Ficoll-Paque gradient and incubated with [5-\( ^{3} \)H]tiazofurin (10 and 100 \( \mu \)M) for periods up to 2 h. The cells were then extracted with 10% trichlo-roacetic acid and neutralized immediately with tri-\( \text{n-} \)octylamine in Freon, and an aliquot was analyzed by high-pressure liquid chromatography using a buffer system of ammonium phosphate (9). The bone marrow and peripheral blood smears were stained with Wright-Giemsa.

Drug and Administration. The tiazofurin in sterile, injectable form was obtained through courtesy of Dr. Jean L. Grem of the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The assistance of Dr. Grem in the escalation schedule of tiazofurin administration is much appreciated.

All drug doses were administered over a period of 1 h by an infusion pump (Imed Co., San Diego, CA) under sterile conditions with nurse and patient wearing face masks. The initial dose of tiazofurin was 1100 mg/m\(^2\) given on Days 1 and 3. Because of lack of effect on GTP concentrations, the dose was increased to 1650 mg/m\(^2\) on Days 4, 5, and 6; the dose was further escalated to 2200 mg/m\(^2\) on Days 7, 8, and 9 and finally to 3300 mg/m\(^2\) on Days 11, 12, and 13. The total dose of tiazofurin administered was 23,650 mg/m\(^2\) (42.6 g) over a period of 13 days, as indicated in Fig. 1.

Informed Consent. Informed consent was obtained according to the principles of the Declaration of Helsinki. The protocol had been reviewed and approved by the Human Experimentation Committee of the Indiana University School of Medicine.

RESULTS

Biochemical and Hematological Impact of Tiazofurin Treatment. The two induction doses of 1100 mg/m\(^2\) yielded no biochemical change in GTP concentration, and the WBC and blast cell count continued to rise. However, 1650 mg/m\(^2\) sharply depressed the GTP concentration in the blast cells to 15%, and subsequently the WBC and blast cell count started to decline (Fig. 1). Two further infusions of 1650 mg/m\(^2\) resulted in keeping the GTP concentrations at 10 to 20% of control. After the third 1650-mg/m\(^2\) dose, the GTP level and the blast count began to rise, and a further dose escalation was necessary. Three daily doses of 2200 mg/m\(^2\) slowly reduced the GTP concentration with some fluctuations eventually to 10 to 15% of control. With a decline in blast cell count after the third 2200-mg/m\(^2\) dose, the blast count and the GTP concentration decreased further; however, after 48 h there was a sharp rebound in the GTP concentration to 80% of control, with no further decrease in blast cell count. Because of the essential absence of toxicity, in order to decrease GTP concentrations and to achieve a further decline in blast cell count, an escalation in dose was instituted. Three subsequent daily treatments of 3300 mg/m\(^2\) reduced the GTP concentrations to the 20% range. Following discontinuation of tiazofurin treatment, the GTP concentration fluctuated from 20 to 50% but declined to the 10% range 3 days after the last injection. This was accompanied by a continued depression of the blast cell count (Fig. 1). By contrast, the WBC cell count decrease was less pronounced, and the patient never became markedly neutropenic during the treatment. The nadir granulocyte count was 1.7 x 10\(^9\) /liter.

Fig. 1. WBC, blasts, and GTP were measured as described in "Materials and Methods" and expressed as the percentage of initial values. At the start of tiazofurin treatment the number of WBC and blast cells was 32.6 and 25.5 x 10\(^9\) cells/liter of blood, respectively. The GTP concentration at the beginning of treatment was 241.8 pmol/10\(^9\) blast cells.

The conversion of radiolabeled tiazofurin to TAD in peripheral leukocytes was examined in vitro prior to therapy and 3 days after cessation of treatment, and the results showed that blast cells accumulated TAD in the magnitude reported in the murine and human tumor cells sensitive to tiazofurin (4, 10).

In the bone marrow, the percentage of decrease in GTP concentration in the blast cells during and after treatment was similar to the percentage of decrease found in the peripheral blast cells (data not shown). During treatment, thrombocytopenia persisted and became more pronounced. Prophylactic platelet transfusions were given on Days 11 and 16 because the platelet count dropped to less than 20 x 10\(^9\) /liter. The patient also received RBC transfusions on Days 5 and 10.

Effect of Tiazofurin on the Differentiation Status of Bone Marrow Cells. Bone marrow aspirations were performed before, during, and after treatment. The results are summarized in Table 1. The aspirates remained very hypercellular with a pronounced decrease in megakaryocytes and erythroblasts. The myeloid series, however, showed a marked shift from myeloblasts before treatment to promyelocytes, myelocytes, metamyelocytes, and bands after treatment. The promyelocytes and the more mature elements, however, were hypogranular and dysplastic.

Toxicity. The patient developed mild itching and a rash on the neck on the first day of treatment which persisted but did not worsen during therapy. Mild conjunctivitis was noted on Day 10 which responded well to dexamethasone eyedrops. Initially, a slight increase in liver function abnormalities was observed: alkaline phosphatase increased to a maximum of 309 units/liter; maximum SGPT was 72 units/liter; maximum SGOT was 93 units/liter (normal, 25 to 45). Bilirubin remained normal. No other side effects were seen except for two episodes of fever in time related to transfusion of blood products. The patient was placed on prophylactic allopurinol therapy, but no hyperuricemia was seen.

Treatment with tiazofurin was discontinued after the third dose of 3300 mg/m\(^2\) to allow for evaluation of possible late side effects. No late toxicity was observed. Tiazofurin was resumed at the same dose after 10 days because of increase in WBC, blasts, and GTP levels in the blasts. To date, more than 3 mo after the start of tiazofurin treatment, the patient is still in good clinical condition on intermittent administration of tiazofurin, although the dose had to be escalated gradually to 6600 mg/
m². No obvious toxicity has been encountered, and his disease is still well controlled.

DISCUSSION

Major disadvantages of current antineoplastic agents in AML are the lack of selectivity in their toxic effect and the absence of reliable and rapid tests to predict sensitivity of the leukemic cells to the therapeutic agents. Based on the predictive test of Jayaram et al. (9), a trial was initiated with tiazofurin in a patient with refractory AML in a relapse. This patient was extensively pretreated with anthracyclines, conventional and high-dose cytarabine, amsacrine, etoposide, and asparaginase, and he had experienced severe toxicity (infections and cerebellar problems). This sharply contrasts with the lack of toxicity during this trial and with earlier reports of Phase I trials with tiazofurin in patients with solid tumors (11-13). Even 3300 mg/m² were well tolerated; it was decided to stop tiazofurin administration to avoid possible late toxicity; however, no toxicity was observed during the 10 days after the discontinuation of the treatment. It is significant that the patient never became markedly neutropenic.

The posttreatment bone marrow showed a marked shift to more mature myeloid elements; these more mature cells, however, had morphological abnormalities, as seen in myelodysplastic bone marrows. No increase in the number of erythroblasts or megakaryocytes was observed. The bone marrow aspirates remained hypercellular during and at the end of tiazofurin treatment. These data suggest in vitro induction of differentiation by tiazofurin and are in keeping with reports providing in vitro evidence that tiazofurin is a potent inducer of differentiation of HL-60 cells (14, 15). Cytogenetic analysis to support this concept of induction of differentiation could not be used in this patient, since the karyotype was normal before therapy and remained normal after therapy. Therefore, it cannot be excluded that the bone marrow aspirate after therapy was a reflection of partial eradication of the leukemic clone combined with partial restoration of normal hematopoiesis.

The lack of toxicity was attributed to our slow administration of the drug by pump over a 1-h period; this apparently saturated receptor sites for TAD on the IMP dehydrogenase of the blast cells as evidenced by the decreased GTP concentrations. With the rapid excretion known to exist for this drug, tiazofurin metabolites either were not formed in many organs or they were degraded; thus the time period or the drug levels were not sufficient to develop toxicity in the brain or elsewhere. Our 1-h infusion method is in contrast with the protocols used by others which did lead to toxicity, i.e., bolus injections (10 min) or round-the-clock 5-day infusion. In the bolus injection, toxic effects might emerge because of the sudden high tiazofurin level achieved which may pass through the blood-brain barrier and may also affect various organs; this apparently did not occur with the 1-h infusion. The 5-day infusion schedule provides a sustained steady-state level of tiazofurin concentration which apparently allows the drug and its metabolites to reach toxic levels in the brain and elsewhere. Future measurements of blood tiazofurin concentrations should test these ideas. The present conspicuous lack of clinical toxicity achieved by our 1-h infusion protocol must be interpreted with caution because of the considerable interpatient variability in tiazofurin toxicity noted in other Phase I trials.

Our observations that the hematological effect was predictable by in vitro testing and that blast cell count followed the decrease in GTP concentrations underline the clinical usefulness of the biochemical predictive test used (9). From the data obtained in this patient, it appears that the degree of GTP depletion required for in vivo activity is >70%. We consider the findings reported here encouraging; they need further testing in a large group of patients.

In the present study tiazofurin was administered as a 1-h perfusion which resulted in a decrease in leukemic blasts. The reduction in leukemic blast count correlated well with the decrease in GTP concentration. Our Phase I and II protocol, approved by the National Cancer Institute, mandated stopping the tiazofurin after 11 doses to avoid possible delayed toxicity. During the following 10-day period, GTP pools and blast cell counts increased to 58 and 40% of pretreatment value, respectively, without any toxicity. After tiazofurin treatment the duration of suppression of GTP pools was 42 h after 2200 and 3300 mg/m². Thus, the drug could be administered once in 2 days after induction of a nadir in GTP concentration (10 to 20% of pretreatment value). Our in vitro predictive test, measuring the accumulation of TAD from labeled tiazofurin in bone marrow or peripheral samples before treatment, should indicate the sensitivity of patient’s leukemic cells to tiazofurin therapy.

If the lack of toxicity with this mode of administration is confirmed then the issue can be addressed as to how to optimize treatment in terms of frequency and duration of tiazofurin therapy based on both nucleotide levels and hematological parameters.

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REFERENCES

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