Biochemically Directed Therapy of Leukemia with Tiazofurin, a Selective Blocker of Inosine 5'-Phosphate Dehydrogenase Activity

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ABSTRACT

Tiazofurin (2-3-d-ribofuranosylthiazole-4-carboxamide, NSC 286193), a selective inhibitor of the activity of IMP dehydrogenase (EC 1.1.1.205), the rate-limiting enzyme of de novo GTP biosynthesis, provided in end stage leukemic patients a rapid decrease of IMP dehydrogenase activity and GTP concentration in the blast cells and a subsequent decline in blast cell count. Sixteen consecutive patients with end stage acute nonlymphocytic leukemia or myeloid blast crisis of chronic granulocytic leukemia were treated with tiazofurin. Allopurinol was also given to inhibit xanthine oxidase activity to decrease uric acid excretion and to elevate the serum concentration of hypoxanthine, which competitively inhibit the activity of hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8), the salvage enzyme of guanylate synthesis. Assays of IMP dehydrogenase activity and GTP concentration in leukemic cells provided a method to monitor the impact of tiazofurin and allopurinol and to adjust the drug doses. In this group of patients with poor prognosis, five attained a complete hematological remission and one showed a hematological improvement. A marked antileukemic effect was seen in two other patients. All five evaluable patients with myeloid blast crisis of chronic granulocytic leukemia reentered the chronic phase of their disease. Five patients with acute nonlymphocytic leukemia were refractory to tiazofurin and three were unevaluable for hematological effect because of early onset of complications. Responses with intermittent 5- to 15-day courses of tiazofurin lasted 3–10 months. Tiazofurin had a clear antiproliferative effect, but the pattern of hematological response indicated that it appeared to induce differentiation of leukemic cells. In spite of toxicity with severe or life-threatening complications in 11 of 16 patients, tiazofurin was better tolerated in most patients than other antileukemic treatment modalities and provided a rational, biochemically targeted, and biochemically monitored chemotherapy which should be of interest in the treatment of leukemias and as a paradigm in enzyme pattern-targeted chemotherapy.

INTRODUCTION

Conventional antileukemic chemotherapy in relapsed or refractory ANLL patients is seldom curative and remissions, if attained, are almost always of short duration. Myeloid blast crisis of CGL is even more resistant to chemotherapy, with complete hematological remission rates varying from 4 to 30% and median survivals being 6–29 weeks (1–4). A primary goal of antileukemic therapy in these patients with poor prognosis should be the identification of agents that are more selective and better targeted in their actions. Such therapy should be based upon our increasing understanding of the biology, enzymology, and biochemistry of cancer cells (5, 6).

In various cancer cells there is a marked enzymic imbalance in purine metabolism, with strong evidence indicating up-regulation of the capacity for guanylate biosynthesis (5, 6). Because the activity of IMPDH, the rate-limiting enzyme of GTP biosynthesis, was increased in all examined cancer cells, it was suggested that IMPDH should be a sensitive target of chemotherapy (5–7). On the basis of this argument, a number of compounds were prepared to block IMPDH activity, among them tiazofurin (8), a C-nucleoside that is metabolized in two enzymic steps to thiazole-4-carboxamide adenine dinucleotide, an NAD analogue that selectively blocks IMPDH activity (9, 10). Tiazofurin in tissue culture and in the murine system potently inhibited IMPDH activity, depressed GTP and dGTP concentrations, and yielded cytotoxicity and inhibition of tumor cell proliferation (10, 11). Recent evidence indicated that, in addition to an increase in IMPDH activity, the activity of the guanine salvage enzyme HGPRT was also elevated in human leukemic cells (12–14). When human myeloid leukemic cells were incubated with radiolabeled tiazofurin, over 20-fold higher concentrations of thiazole-4-carboxamide adenine dinucleotide were produced than in bone marrow cells from healthy volunteers (15, 16). These results provided support for the consideration of tiazofurin in the treatment of myelocytic leukemia. Inhibition of IMPDH activity by tiazofurin in blast cells should decrease GTP and dGTP concentrations, which should restrain the biosynthesis of DNA. Moreover, the blocking of guanylate biosynthesis might critically limit the availability of GTP for expression of the ras oncogene, G protein function, and the biosynthesis of specific proteins (Fig. 1). This approach was strengthened by the observation that the guanine-salvaging activity of HGPRT can be competitively inhibited by hypoxanthine and that the concentration of hypoxanthine can be markedly increased in the plasma by allopurinol, originally given to block xanthine oxidase activity to decrease uric acid excretion (14). Therefore, to attain an optimal impact leading to depletion of GTP in the blast cells, simultaneous administration of tiazofurin and allopurinol is essential. Since the action of tiazofurin and allopurinol is well characterized, the biochemical impact of these drugs can be monitored during treatment by frequently sampling leukemic cells for measurement of IMPDH activity and GTP concentrations.

This paper reports our biochemically directed trial in patients with ANLL and myeloid CGL-BC, where tiazofurin rapidly brought down IMPDH activity and then GTP concentrations, followed by a decline in the blast cell count.

MATERIALS AND METHODS

Patients. Sixteen consecutive patients were treated with tiazofurin at Indiana University Medical Center from March 1987 to April 1988. The characteristics of the patients are listed in Table 1. This Phase I/II trial was monitored by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (Bethesda,
MD). Patients reported on here suffered from refractory or relapsed ANLL, treatment-related ANLL, ANLL after a myelodysplastic syndrome, or nonlymphoid blast crisis of CGL, based on morphology and immunological markers (negative for TdT and the common acute lymphocytic leukemia antigen and positive for at least one of the myeloid markers, VIM-2, My 9, and My 7). Eligibility criteria required a first remission duration of <12 months; however, the initial remission duration in patients 1 and 8 was 18 and 13 months, respectively. Patient 11 on admission had serum bilirubin >1.5 mg/dl. The initial clinical course of the first patient studied (patient 9) was published (16).

Evaluation of Response. A complete remission was defined by standard criteria (17). Hematological improvement was defined as a bone marrow blast count of <5% within the peripheral blood, absolute granulocyte count of >1.0 x 10^9/liter, and a platelet count of >50 x 10^9/liter (18). Antileukemic effect was defined as a complete disappearance of all blasts from the peripheral blood with a bone marrow blast count of <5%. Patients who did not meet these criteria were considered nonresponders.

Evaluation of Toxicity. Toxicity was evaluated according to the criteria of the Eastern Cooperative Oncology Group (19).

Informed Consent. Informed consent was obtained from all patients according to the principles of the Declaration of Helsinki. The protocol had been reviewed and approved by the Investigational Review Board of the Indiana University School of Medicine.

Biochemical Assays. Preparation of bone marrow and peripheral blood mononuclear cells and determination of the concentration of GTP were carried out as reported (15). Briefly, mononuclear cells were separated by a Ficoll-Paque density gradient. An aliquot of cells was then extracted with 10% trichloroacetic acid, neutralized immediately with tri-n-octylamine in Freon, and analyzed by high pressure liquid chromatography for GTP concentrations, using a buffer system of ammonium phosphate. Another aliquot of cells was assayed directly for IMPDH activity as described (20). During treatment with tiazofurin, IMPDH activity and GTP pools in leukemic cells were measured at least 3 times/day in peripheral blood samples.

Chromosome Preparation and Analysis. Cytogenetic investigations were performed on bone marrow or peripheral blood using direct analysis, short term incubation for 24 h, or culturing without phytohemagglutinin for 48 h. Chromosome preparations were studied at ≥400-band level (21).

Drug and Administration. Tiazofurin in sterile injectable form was obtained from the National Cancer Institute (Bethesda, MD). The drug was administered daily, over a period of 1 h, by an infusion pump (Imed Co., San Diego, CA) under sterile conditions. Uniformity in treatment protocol was provided by biochemical targeting to decrease GTP concentrations to <20% of control; therefore, individual schedules were required, involving variations in tiazofurin dose. In the first patient in the study (patient 9), the initial dose was 1100 mg/m², based on data from previous Phase I trials. This dose was too low to decrease GTP levels. Therefore, all subsequent patients were initially given 2200 mg/m² (an exception was patient 1, who received 5800 mg/m² the first day because of a mistake made in the hospital pharmacy). The different timings of dose escalations were based on the biochemical response; the goal was to avoid unnecessary dose escalations. If an insufficient decline occurred in GTP levels, further dose escalations were initiated. At the end of the study, we concluded that the best results are obtained by escalating uniformly after 3 days in the case of lack of decrease in GTP concentration to <20% and that each treatment course should be no longer than 15 days.

Tiazofurin at the effective dose was then administered until the peripheral blood was cleared of blast cells. This pattern of administration was followed in all the patients except for the first one (patient 9) who was treated with tiazofurin for only 5 days at the effective dose. Patients who attained a complete remission or hematological improvement received further courses of tiazofurin as consolidation treatment (patient 1) or whenever an increase in absolute numbers of blast cells (>5 x 10^9/liter) was noted in the peripheral blood (patients 2-6 and

![Fig. 1. Pathways of IMP and GTP metabolism and the biochemical basis for the chemotherapeutic impact of tiazofurin and allopurinol. Guo, guanine; GPRT, guanine phosphoribosyltransferase.](image-url)

Table 1 Patients' characteristics, total dose and number of days of initial tiazofurin treatment, outcome, and duration of response

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/Age</th>
<th>Disease</th>
<th>Status</th>
<th>Total dose of tiazofurin (mg/m²)</th>
<th>No. of days</th>
<th>Outcome</th>
<th>Response duration (mo)</th>
<th>Total survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F/53</td>
<td>ANLL</td>
<td>First relapse</td>
<td>19,000</td>
<td>7</td>
<td>Complete remission</td>
<td>10</td>
<td>15</td>
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<tr>
<td>2</td>
<td>M/27</td>
<td>CGL</td>
<td>Myeloid blast crisis</td>
<td>41,800</td>
<td>11</td>
<td>Complete response</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>F/20</td>
<td>CGL</td>
<td>Myeloid blast crisis</td>
<td>22,000</td>
<td>8</td>
<td>Complete response</td>
<td>6</td>
<td>6*</td>
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<tr>
<td>4</td>
<td>M/44</td>
<td>CGL</td>
<td>Myeloid blast crisis</td>
<td>24,200</td>
<td>11</td>
<td>Complete response</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>5</td>
<td>M/56</td>
<td>CGL</td>
<td>Myeloid blast crisis</td>
<td>46,200</td>
<td>6</td>
<td>Complete response</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
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<td>F/58</td>
<td>CGL</td>
<td>Myeloid blast crisis</td>
<td>20,900</td>
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<td>Hematological improvement</td>
<td>10</td>
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<td>Secondary leukemia</td>
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<td>&lt;1</td>
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<td>M/55</td>
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<td>First relapse</td>
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<td>26</td>
<td>Antileukemic effect</td>
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<td>&lt;1</td>
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<tr>
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<td>9</td>
<td>M/48</td>
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<td>Second relapse</td>
<td>23,650</td>
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<td>No response</td>
<td>4</td>
<td></td>
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<tr>
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<td>F/21</td>
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<td>2</td>
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<td>11</td>
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<td>Refractory first relapse</td>
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<td>M/24</td>
<td>ANLL</td>
<td>First relapse</td>
<td>27,500</td>
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<td>No response</td>
<td>2</td>
<td></td>
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<tr>
<td>13</td>
<td>M/41</td>
<td>ANLL</td>
<td>First relapse</td>
<td>20,900</td>
<td>10</td>
<td>No response</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Unvaluable for hematological effect</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>M/83</td>
<td>ANLL</td>
<td>Post MDS</td>
<td>14,300</td>
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<td>&lt;1</td>
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<tr>
<td>15</td>
<td>F/50</td>
<td>ANLL</td>
<td>Post MDS</td>
<td>5,500</td>
<td>3</td>
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<td>8</td>
<td></td>
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<tr>
<td>16</td>
<td>M/47</td>
<td>ANLL</td>
<td>Myeloid blast crisis</td>
<td>2,200</td>
<td>1</td>
<td>Unvaluable</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

* Patient returned to primary physician, in California, who treated her there with mitoxanthrone to prepare her for bone marrow transplantation, but patient died during mitoxanthrone chemotherapy.
During these intermittent courses, the dose of tiazofurin was escalated in some patients to 6600 mg/m², if an inadequate decline in GTP levels was seen.

On admission, a permanent central line was placed in all patients. When neutropenic, patients received antimicrobial prophylaxis with norfloxacin and ketoconazole. While receiving tiazofurin, all patients were placed on allopurinol, 300 mg/day or 100 mg every 4–6 h. Allopurinol was given initially to prevent hyperuricemia (300 mg/day). Subsequently, divided doses of allopurinol (100 mg, 4 to 8 times/day) were given in an attempt to maintain high and more constant hypoxanthine levels in the serum. Depletion of GTP in blast cells depended on both tiazofurin and allopurinol, to block the de novo and salvage pathways, respectively.

RESULTS

Biochemical Parameters during Tiazofurin Treatment. A correlation between biochemical and hematological responses was seen in all patients. Figs. 2 and 3 are typical of positive responses; they were absent when clinical treatment failed. Institution of tiazofurin therapy resulted in a rapid decrease in IMPDH activity. The decline in GTP pools occurred more slowly, and dose escalations were necessary in most patients to reach a >80% decline. The decline in GTP pools was followed by a rapid decrease in the absolute numbers of peripheral WBC and blast cells. In patients 11 and 12, who were refractory to tiazofurin treatment, IMPDH activity remained elevated and GTP pools were never depressed to <20% of pretreatment values. Patients 1–6 and 9 received multiple courses of tiazofurin treatment. Clinical relapse of the disease in patients 2–6 and 9 was always associated with marked increases in IMPDH activity and GTP pools in the leukemic cells. Reinstitution of tiazofurin (Fig. 3) was accompanied by a biochemical and hematological response similar to that observed during the initial treatment. This pattern was seen with all retreatment courses except during the fifth course of treatment in patient 9; he became refractory to tiazofurin by both biochemical and hematological parameters.

Clinical Response to Tiazofurin. Outcome of treatment and duration of response are provided in Table 1. Peripheral blood cell counts obtained from responders prior to, at the end of, and 7–10 days after treatment are given in Table 2. Bone marrow cellularity, bone marrow cell differential counts, and cytogenetic analyses of these responders are shown in Table 3.

Five patients, one with ANLL and four with CGL-BC, attained a complete hematological remission. The patient with ANLL also entered a cytogenetic remission, while chromosome abnormalities persisted in the patients with CGL-BC. One patient with CGL-BC showed a hematological improvement. In two patients with ANLL a marked antileukemic effect was seen; both patients died, however, from invasive fungal infections. Five patients were classified as therapy failures, although some antileukemic effect was seen in three of these (patients 9–11), with a >75% decrease in the absolute number of peripheral blasts in all three and a >75% decrease in bone marrow blasts in patient 9 (16).

The hematological effect of tiazofurin was not evaluable in three patients because of early severe or life-threatening toxicity, consisting of seizures (patient 14), pleuroperticarditis (patient 15), and sudden coma (patient 16).

Most of the responders maintained a granulocyte count of >0.5 x 10⁹/liter during and after treatment. Patients 1, 3, and 4 showed an increase in platelet counts after tiazofurin treatment.

Bone marrow specimens obtained during and shortly after treatment remained normo- or hypercellular in all responders except patients 4 and 8 (Table 3). Posttreatment bone marrow aspirates in responders showed a shift from blast cells to more mature cells, except in patient 8, who received 25 days of tiazofurin and became severely hypoplastic after treatment.

The complete remission in patient 1 lasted for 10 months. The duration of response in the CGL-BC patients was 3, 6, 6, 7, and 10 months. Patient 2 became clinically refractory to treatment after 3 months; at that time, he had developed an additional chromosome abnormality. After four cycles of good response, patient 3 discontinued treatment to undergo an allogeneic bone marrow transplantation with an unrelated donor. Patients 4–6 remained clinically and biochemically sensitive to tiazofurin but preferred to be switched to oral antileukemic therapy. Two of these patients were refractory to hydroxyurea and 6-thioguanine before they began to be treated with tiazofurin; retreatment with these agents resulted in an adequate control of peripheral WBC and blast count.

Toxicity to Tiazofurin. Adverse events observed in the 16 patients treated with tiazofurin are listed in Table 4. All adverse events that occurred during treatment or within 4 weeks after cessation of the therapy with tiazofurin are listed, irrespective of the fact that some of these toxicities might have been caused or aggravated by concomitant therapy or by the patient’s un-
TIAZOFURIN TREATMENT OF LEUKEMIA

The values of WBC, blast cells, GTP pools, and IMPDH activity are expressed as a percentage of pretreatment values. The pretreatment value of WBC was 6.6 x 10^9/liter; blast cells, 4.0 x 10^9/liter; GTP pools, 344.3 nmol/10^9 leukemic cells; and IMPDH activity, 12.2 nmol/h/mg protein.

Table 2 Effect of tiazofurin therapy on peripheral blood counts of patients who responded to tiazofurin therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>WBC (x10^9/liter)</th>
<th>Blasts (x 10^9/liter)</th>
<th>Granulocytes (x 10^9/liter)</th>
<th>Platelets (x 10^9/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre°</td>
<td>End*</td>
<td>Post'</td>
<td>Pre°</td>
</tr>
<tr>
<td>1</td>
<td>1.3</td>
<td>1.5</td>
<td>4.0</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>148.0</td>
<td>22.1</td>
<td>4.0</td>
<td>40.0</td>
</tr>
<tr>
<td>3</td>
<td>6.6</td>
<td>2.4</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>12.6</td>
<td>1.5</td>
<td>3.2</td>
<td>1.0</td>
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<td>5</td>
<td>94.0</td>
<td>9.8</td>
<td>1.6</td>
<td>19.7</td>
</tr>
<tr>
<td>6</td>
<td>19.0</td>
<td>5.4</td>
<td>4.1</td>
<td>7.4</td>
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<td>7</td>
<td>111.3</td>
<td>7.5</td>
<td>2.6</td>
<td>46.5</td>
</tr>
<tr>
<td>8</td>
<td>17.4</td>
<td>0.4</td>
<td>0.7</td>
<td>12.7</td>
</tr>
</tbody>
</table>

* Prior to the institution of therapy.
* On the day therapy was discontinued.
* 7–10 days after discontinuation of therapy.
* Patient receiving platelet transfusions.

Table 3 Bone marrow findings before and after tiazofurin treatment in the eight responders

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cellularity*</th>
<th>Blasts (%)</th>
<th>Pro® + Myelo (%)</th>
<th>MM + Gran (%)</th>
<th>Cytogenetics</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>1</td>
<td>Hypo</td>
<td>Normal</td>
<td>36%</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
<td>Hyper</td>
<td>Hyper</td>
<td>NE</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Hyper</td>
<td>Hyper</td>
<td>55</td>
<td>4</td>
<td>29</td>
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<tr>
<td>4</td>
<td>Hyper</td>
<td>Hypo</td>
<td>33</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>Hyper</td>
<td>Normal</td>
<td>48</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>Hyper</td>
<td>Hypo</td>
<td>60</td>
<td>1</td>
<td>12</td>
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<tr>
<td>8</td>
<td>Normal</td>
<td>Hypo</td>
<td>82</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cellularity was estimated in bone marrow biopsies or aspirates.
® Pro, promyelocytes; Myelo, myelocytes; MM, metamyelocytes; Gran, granulocytes; NE, not evaluable because of dry tap; ND, not done.
® Percentage of cells on a 200-cell marrow differential of a Wright-Giemsa-stained bone marrow aspirate specimen.

underlying hematological disorder. Most of the toxic events (66%) were mild or moderate. The most common side effect was drowsiness. Patients with drowsiness were easily arousable and were fully awake by the time the next infusion was given. Other mild and moderate toxicities noted were nausea, vomiting, headaches, myalgias, conjunctivitis, skin rash, and atrial fibrillation.

Five patients developed clinical signs of pleuropéricarditis. In all five patients, pain due to the serositis improved within 48 h after discontinuation of tiazofurin and institution of corticosteroids. Chest X-rays and echocardiograms remained normal. One patient had electrocardiogram changes suggestive of pericarditis; in another patient the pericarditis was associated with atrial fibrillation requiring digitalization. Tiazofurin therapy could be resumed without reappearance of clinical symptoms of pleuropéricarditis in three patients.

Patient 12 developed a pericardial friction rub during his second course of tiazofurin; he quickly improved on corticosteroids and tiazofurin therapy was restarted. However, 9 days after the completion of treatment, he was readmitted to the hospital with fever, skin lesions, swelling of both knees, severe neutropenia, and progressive leukemia. A pericardial friction rub was noted. Echocardiogram performed 5 days later showed a large pericardial effusion and the patient died from the consequences of cardiac tamponade. Patient 15 developed acute chest pain and shortness of breath after 3 days of tiazofurin. She recovered completely but received no further treatment. Three patients developed life-threatening infections. Patients 7

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and 8 developed invasive fungal infections and patient 14 an
aspiration pneumonia. Patients 6 and 14 developed seizures
which were difficult to control and they needed multiple anti-
convulsant drugs. Patient 6 recovered completely and tiazofurin
therapy could be restarted; she experienced a second episode of
seizures at a higher dose of tiazofurin. Patient 14 remained
somnolent and confused and subsequently died of an aspiration
pneumonia.

Two patients showed a marked increase in liver function
abnormalities. Patient 8 was being given broad spectrum antibi-
totics, acyclovir, and amphotericin B for septicemia and esoph-
agitis with Candida albicans and herpes simplex; patient 11
already had impaired liver function before the start of tiazo-
furin.

Two patients developed sudden coma. Patient 11 had an
infection, was on broad spectrum antibiotics, and was com-
pletely refractory to platelet transfusions. Patient 16 was ad-
mitted with a WBC count of 282 × 10^9/liter and 79% blasts.
He presented with clinical findings consistent with a hyperleu-
cytocytic syndrome. He was subjected to leukapheresis twice and
tiazofurin therapy was then started. He abruptly developed
coma with signs of decerebration, intractable hypotension, and
respiratory failure, 6 h after the completion of the first dose of
tiazofurin. He died the next day; autopsy was refused. Two
patients experienced ventricular fibrillation and cardiac arrest
(patients 8 and 11).

DISCUSSION

The results of this Phase I/II trial with tiazofurin in leukemia
are very encouraging because five patients (31%) attained a
complete remission, one (6.5%) had a hematological improve-
ment of less than 15 days and without major complicating medical problems at entrance in the protocol; group B, 5 patients treated for
longer than 15 days or entering trial with major complicating medical problems.

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>Mild A</th>
<th>B</th>
<th>Moderate A</th>
<th>B</th>
<th>Severe A</th>
<th>B</th>
<th>Life-threatening A</th>
<th>B</th>
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<tbody>
<tr>
<td>Drowsiness, confusion</td>
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It is yet unknown whether tiazofurin acts solely as an anti-
proliferative agent or whether it may also induce maturation of
leukemic cells. Observations from an ANLL patient who achieved complete remission with loss of her marker chromo-
somes and from one patient who developed severe marrow
hypoplasia after prolonged tiazofurin therapy provide us with
clinical evidence that this drug clearly has an antiproliferative
effect. In contrast, the absence of bone marrow hypoplasia, the
marked shift from blast cells to mature granulocytes in the bone
marrow, the persistence after the cessation of tiazofurin admin-
istration of adequate numbers of granulocytes and platelets,
and the persistence of chromosome abnormalities observed in
the other responding patients provide evidence that this drug
promoted leukemic cell differentiation in vivo (Table 3). This
suggestion is supported by in vitro studies with tiazofurin in
human leukemic cell lines (23–25). However, the possibility
that tiazofurin induces maturation of leukemic cells in vivo
must be substantiated by further clinical investigations. Toxici
has been well described in Phase I trials with tiazo-
urin (26–31). Toxicity was also substantial in this trial. The
most severe complications, however, such as sudden coma, ven-
tricular fibrillation, and cardiac tamponade, were seen in
patients with either complicating medical problems such as
hyperviscosity syndrome, abnormal liver function, and progres-
severe hypoplasia or prolonged treatment with tiazofurin (over 15
days), resulting in bone marrow hypoplasia with infectious
complications. The exact contribution of tiazofurin to these
severe toxicities is therefore difficult to determine. As Table 4
shows, toxicity was lower in patients who entered the study
with few complications and were treated for less than 15 days
in each cycle. More severe and life-threatening toxicity occurred
in patients who entered in a severely compromised status and
were treated longer than 15 days in the cycles. In future trials,
we would recommend the restriction of tiazofurin treatment to
patients in stable clinical condition without complicating medical problems; the total duration of treatment should not exceed 10 to 15 days/course. Drowsiness was quickly reversible after discontinuation of therapy and never resulted in medical problems. Myalgias and pleuropertocarditis rapidly responded to temporary discontinuation of the tiazofurin and/or institution of prednisone therapy; myalgias and pleuropertocarditis did not prevent further therapy with tiazofurin. Seizures were preceded by hypertension; since we promptly instituted antihypertensive therapy with calcium channel blockers in patients with hypertension, no new seizures have been observed.

Novel aspects of this study include the following. (a) The clinical trial was rationally targeted against the enzyme pattern of elevated IMPDH and HGRT activity in the leukemic blast cells. (b) Infusion of tiazofurin rapidly decreased IMPDH activity and curtailed GTP concentrations in the blast cells, yielding a clearing of blast cells (c) Measurement of the behavior of these biochemical targets provided monitoring of therapeutic progress and allowed adjustment of frequency and dose of administration of tiazofurin and allopurinol. (d) Allopurinol not only was used as a blocker of uric acid formation but also was targeted to raising serum hypoxanthine concentrations to inhibit HGRT activity.

Tiazofurin appears to be a novel promising agent in the treatment of leukemia, if toxicity can be better handled. A larger number of patients will have to be studied to confirm its activity. Future studies should aim at improving efficacy by combining tiazofurin with other antileukemic agents or inducers of differentiation.

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REFERENCES

Biochemically Directed Therapy of Leukemia with Tiazofurin, a Selective Blocker of Inosine 5′-Phosphate Dehydrogenase Activity

Guido J. Tricot, Hiremagalur N. Jayaram, Elizabeth Lapis, et al.


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