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# SELECTIVE SENSITIVITY TO TIAZOFURIN OF HUMAN LEUKEMIC CELLS*

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**Abstract**—This study reports the selective sensitivity to tiazofurin (2-beta-D-ribofuranosylthiazole-4-carboxamide, NSC-286193) of human leukemic leukocytes as compared to normal ones in bone marrow and peripheral blood samples by comparing the production of the active metabolite, thiazole-4-carboxamide adenine dinucleotide (TAD), from labeled tiazofurin and the depression of GTP concentration. When labeled tiazofurin was incubated with leukocytes obtained from healthy volunteers or from leukemic patients (acute non-lymphocytic leukemia or acute lymphoblastic leukemia), the TAD production was 27.0 ± 8.3, 551.3 ± 71.8 and 755.9 ± 94.1 pmoles/$10^9$ cells per hr, respectively. Thus, the leukemic cells produced over 20-fold higher concentrations of TAD than the normal leukocytes. Incubation with tiazofurin in leukemic leukocytes decreased the GTP pools (to 48–79%), whereas there was no change in the normal leukocytes. These results indicate a selectivity of response to tiazofurin in human normal and leukemic leukocytes. The procedure reported in this work may be suitable as a rapid predictive test for the sensitivity of leukemic leukocytes to tiazofurin. Such a diagnostic test should be helpful in identifying neoplastic cells sensitive to tiazofurin in the Phase II trials now being developed.

Tiazofurin§ (2-β-D-ribofuranosylthiazole-4-carboxamide) exhibits potent antitumor activity against murine tumors [1, 2] and is now undergoing Phase I trials [3, 4]. The mechanism of action of tiazofurin is due to its anabolism to thiazole-4-carboxamide adenine dinucleotide (TAD), an analog of NAD wherein nicotinamide is replaced by thiazole-4-carboxamide [5]. TAD potently inhibits IMP dehydrogenase activity, causing depression of guanylate (GMP, GDP, GTP and dGTP) pools leading to inhibition of tumor cell proliferation [6, 7]. The present study was aimed at identifying human neoplastic cells sensitive to tiazofurin action. A test that can predict sensitivity to tiazofurin should facilitate the planning of Phase II trials.

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§ Abbreviations: ALL, acute lymphoblastic leukemia; AMSA, amsacrine; ANLL, acute non-lymphocytic leukemia; ARA-C, cytosine arabinoside; DNR, daunorubicin; HDARA-C, high dose cytosine arabinoside; PRED, prednisone; TAD, thiazole-4-carboxamide adenine dinucleotide; TCA, trichloroacetic acid; 6-TG, 6-thioguanine; tiazofurin, 2-beta-D-ribofuranosylthiazole-4-carboxamide; TRMP, tiazofurin-5′-phosphate; VCR, vincristine; and VP-16, etoposide.

## METHODS

*Chemicals and reagents.* Tiazofurin (NSC-286193) was provided by Dr. Ven Narayanan, National Cancer Institute (NCI), Bethesda, MD. [5-$^3$H]Tiazofurin (1.96 Ci/mmole) was obtained from Research Triangle Institute, Research Triangle Park, NC,

Table 1. Metabolism *in vitro* of tiazofurin in leukocytes of healthy volunteers

| | Metabolism of tiazofurin (pmoles/$10^9$ cells/hr) | | |
|---|---|---|---|
| Sex | TRMP | TAD | Ratio of TAD/TRMP |
| Female | 245.6 | 58.1 | 0.2 |
| Female | 157.8 | 18.2 | 0.1 |
| Female | 232.3 | 13.0 | 0.1 |
| Female | 361.9 | 15.6 | 0.04 |
| Male | 263.6 | 29.9 | 0.1 |
| Means ± S.E. | 252.2 ± 32.8 | 27.0 ± 8.3 | |

Preparation of leukocytes from bone marrow aspirates is described in Methods. Accumulation of TAD was linear for up to 2 hr in these experiments.



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through the courtesy of J. P. Davignon of NCI. The metabolites of tiazofurin (its mono-, di-, and triphosphates and TAD) were synthesized and provided by Dr. V. Marquez of NCI [8]. Ficoll-Paque for *in vitro* cell isolation was obtained from Pharmacia Fine Chemicals, Piscataway, NJ.

*Determination of tiazofurin metabolites.* Bone marrow aspirates (5 ml) from normal healthy volunteers or leukemic patients, or peripheral blood specimens (10 ml) from leukemic patients, were diluted with cold RPMI 1640 medium containing 2 mM L-glutamine (total volume of 25 ml), layered over cold Ficoll-Paque and centrifuged at 500 *g* for 25 min in a cooled (0–5°) Beckman table top centrifuge, model TJ-6R. The leukocyte layer which was well separated from the red cell pellet was carefully transferred to a fresh tube, and 25 ml of the same medium was added. The cells were separated by centrifugation at 750 *g* for 5 min in a Beckman centrifuge (see above) and then washed twice with 25 ml of cold medium. The leukocytes (1 to $10 \times 10^6$ cells/ml) were then suspended in RPMI 1640 medium containing 2 mM L-glutamine and 10% dialyzed fetal bovine serum (K.C. Biological, Lenexa, KS), aliquots of 5 ml/flask were dispensed, and the flasks were preincubated at 37° in an atmosphere of 5% $CO_2$:95% air for 30 min. To the flasks, saline or [5-$^3$H]tiazofurin (10 $\mu$M, 27.8 mCi/mmole) was added and incubated at 37° in an atmosphere of 5% $CO_2$:95% air. After 2 hr of incubation the cell suspension was transferred quickly to centrifuge tubes, the flask was washed once with 5 ml of cold phosphate-buffered saline (PBS), and the washings were added to the centrifuge tubes containing cell suspension and centrifuged at 1500 *g* for 2 min at 0–5°. One milliliter of cold PBS was added to the cell pellet, resuspended in Eppendorf centrifuge tubes, and spun at 12,000 *g* for 0.2 min. The cell pellet was washed once more with 1 ml of cold PBS and then 300 $\mu$l of 10% cold trichloroacetic acid (TCA) was added. The protein-free supernatant fraction was neutralized with 0.5 M trioctylamine in freon [9]. Aliquots of neutralized samples were analyzed on a Partisil 10-SAX column (Waters Associates, Milford, MA) pre-equilibrated with 5 mM ammonium phosphate buffer, pH 3.9. Tiazofurin and its metabolites were eluted using a gradient of 5 mM ammonium phosphate buffer, pH 3.9, to 500 mM ammonium phosphate buffer, pH 3.5 [10]. Under these conditions, tiazofurin, tiazofurin-5′-phosphate (TRMP) and TAD eluted at 3, 12 and 26 min respectively. Metabolite concentrations were quantitated by measuring radioactivity eluting in these peaks.

*Nucleotide concentrations.* Leukocytes (1 to $10 \times 10^6$ cells/ml) obtained by the above procedure were suspended (5 ml) in RPMI 1640 medium containing 2 mM L-glutamine and 10% dialyzed fetal bovine serum and incubated with 100 $\mu$M tiazofurin or saline for 2 hr at 37° in an atmosphere of 5% $CO_2$ and 95% air. Following incubation with the drug, the cells were quickly processed as detailed above for the tiazofurin metabolic studies. An aliquot of neutralized TCA extract was analyzed on a Partisil 10-SAX column as detailed above. Under the conditions of the analysis GTP eluted at 39 min.

Table 2. Clinical profile of leukemic patients

| Types of leukemias* | Age/Sex | Morphologic subtypes* | Peripheral leukocytes | | Bone marrow | | Prior therapy | Interval from last treatment (months) |
|---|---|---|---|---|---|---|---|---|
| | | | Counts/mm$^3$ | Blasts (%) | Cellularity (%) | Blasts (%) | | |
| Acute non-lymphocytic leukemia | 30/M | M-5 | 2,000 | 5 | 40 | 45 | DNR/ARA-C | 14 |
| | 27/M | M-1 | 17,800 | 27 | 95 | 99 | None | |
| | 23/F | M-3 | 13,200 | 4 | 95 | 40 | None | |
| | 77/F | M-1 | 9,460 | 63 | 90 | 90 | None | |
| | 20/M | M-4 | 101,200 | 71 | 50 | 90 | None | |
| | 64/M | M-4 | 30,000 | 80 | 60 | 40 | DNR/ARA-C<br>AMSA/ARA-C | 6 |
| | 61/F | M-2 | 5,600 | 58 | 60 | 95 | DNR/ARA-C/6-TG<br>HDARA-C | 4 |
| | 46/F | M-4 | 46,300 | 43 | 90 | 95 | None | |
| Acute lymphoblastic leukemia | 57/F | L-2 | 135,000 | 97 | 95 | 95 | VCR/DNR/PRED<br>ARA-C/VP-16 | 3 |
| | 21/M | T-cell | 170,000 | 99 | 95 | 99 | None | |

* French/American/British classification.

Table 3. Metabolism *in vitro* of tiazofurin in leukemic leukocytes

| Patient | Cell source | Tiazofurin metabolites (pmoles/$10^9$ cells/hr) TRMP | TAD | Ratio of TAD/TRMP |
|---|---|---|---|---|
| | | Acute non-lymphocytic leukemia | | |
| S. B. | Peripheral | 95.0 | 765.0 | 8 |
| A. M. T. | Peripheral | 215.0 | 775.0 | 4 |
| H. O. C. | Bone marrow | 167.0 | 517.0 | 3 |
| J. L. H. | Bone marrow | 226.6 | 607.0 | 3 |
| C. H. | Bone marrow | 372.5 | 286.8 | 1 |
| C. H. | Peripheral | 281.1 | 279.0 | 1 |
| L. R. O. | Bone marrow | 374.7 | 852.6 | 2 |
| L. G. P. | Bone marrow | 227.8 | 364.8 | 2 |
| L. L. S. | Bone marrow | 230.0 | 515.0 | 2 |
| Mean ± S.E. | | 243.3 ± 30.0 | 551.3 ± 71.8 | |
| | | Acute lymphoblastic leukemia | | |
| R. K. K. | Bone marrow | 342.5 | 661.8 | 2 |
| F. K. | Peripheral | 16.3 | 850.0 | 52 |
| Mean ± S.E. | | 179.4 ± 163.1 | 755.9 ± 94.1 | |

Experimental conditions used are described in Methods.

## RESULTS AND DISCUSSION

*Metabolism of tiazofurin in normal and leukemic leukocytes.* To elucidate the action of tiazofurin in leukocytes from normal and leukemic subjects, the metabolism of this drug was examined first in the leukocytes obtained from healthy volunteers (Table 1). There was a significant concentration of tiazofurin-5′-phosphate, and a small percentage of it (11%) was found as TAD.

The metabolism of tiazofurin was studied in leukocytes obtained from leukemic patients with acute non-lymphocytic leukemia (ANLL) and acute lymphoblastic leukemia (ALL). The patients had received no chemotherapy for at least 3 months prior to study (Table 2). Leukemic leukocytes from bone marrow and peripheral blood of the same ANLL patient were examined for tiazofurin metabolism (Table 3). There was a 10-fold higher concentration of TAD in leukemic (Table 3) than in normal leukocytes (Table 1). Examination of samples obtained from the same ANLL patient (patient C. H.) suggests that there may not be a difference between the TAD content in leukemic leukocytes taken from bone marrow or peripheral blood (Table 3).

The concentrations of metabolites of tiazofurin in the leukocytes from seven ANLL patients are shown in Table 3. In the ANLL leukocytes, there was a 10- to 20-fold higher content of TAD than in normal leukocytes.

Leukocytes from acute lymphoblastic leukemic patients had 10- to 20-fold higher TAD concentrations than leukocytes from normal volunteers (Table 3). The ratio of the amount of TAD/TRMP (2 and 52) indicated an increased concentration of TAD in the lymphoblasts.

Table 4. Effect of tiazofurin incubation on leukocyte GTP pools, *in vitro*

| Patients | Cells | GTP (nmoles/$10^9$ cells) Control | GTP (% control following tiazofurin treatment) |
|---|---|---|---|
| Female | Normal | 421.9 ± 28.0 | 99 |
| Male | Normal | 321.8 ± 2.0 | 104 |
| Mean ± S.E. | Normal | 371.8 ± 15.0 | 101 |
| L. L. S. | ANLL | 272.7 ± 40.3 | 58* |
| L. R. O. | ANLL | 273.0 ± 13.0 | 48* |
| H. O. C. | ANLL | 212.4 ± 3.8 | 58* |
| L. G. P. | ANLL | 240.7 ± 12.0 | 79* |
| A. M. T. | ANLL | 220.0 ± 18.0 | 53* |
| Mean ± S.E. | ANLL | 243.8 ± 17.4 | 59 ± 5 |
| R. K. K. | ALL | 346.0 ± 12.0 | 60* |

Leukocytes were isolated as described in Methods. Leukocytes (5 to 50 × $10^6$ cells) were incubated with 100 μM tiazofurin in RPMI 1640 medium containing 2 mM L-glutamine and 10% dialyzed fetal bovine serum at 37° in an atmosphere of 5% $CO_2$:95% air for 2 hr. GTP content in the cells was determined as detailed in Methods.

* Significantly different from controls ($P < 0.05$).

To examine whether an increased accumulation of TAD in human leukemic leukocytes leads to a decrease in GTP content, normal and leukemic leukocytes were incubated with tiazofurin or physiological saline for 2 hr and the concentration of GTP was measured. As indicated in Table 4, leukocytes from normal volunteers did not have a depression in GTP pools following tiazofurin incubation. The decrease in GTP pools in leukemic cells following tiazofurin incubation was selective in leukemic leukocytes and is in agreement with the increase in TAD concentration leading to decreased guanylate pools. Increased TAD content also led to a reduction in GTP concentration in tiazofurin susceptible murine tumors, and in cultured human leukemic and lung cancer cells [2, 7, 11–13].

The procedure described here with human leukocytes could be applied as a rapid test to determine whether the given leukemic cells are likely to be sensitive or resistant to tiazofurin treatment. The data presented indicate that human ANLL and ALL cells should be sensitive to tiazofurin therapy. Since leukocytes from healthy volunteers had only small amounts of TAD without a reduction in GTP pools, this might indicate selectivity in tiazofurin action against leukemic cells. This appears to be the first study where the TAD content and its action on GTP concentration in neoplastic cells and their appropriate cells of origin have been compared.

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