Pharmacology of 6-Thioguanine in Man

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SUMMARY

6-Thioguanine (6-TG) was administered to 15 patients with acute leukemia and to 9 with solid tumors in 32 experiments, as part of Phase I testing of 6-TG. 6-TG and metabolites were measured by labeling the 6-TG with radiosulfur. The levels of 6-TG and metabolites were measured in blood and urine after p.o. and i.v. dosage. Incorporation into DNA and RNA of the bone marrow was measured. Blood levels of 6-TG were higher after administration of i.v. dosages than after equal p.o. doses were given the same patients. Blood levels and incorporation into bone marrow DNA were higher with the higher doses but depended more upon the growth status of the marrow than upon dose level. Incorporation into DNA of the marrow was usually very small after administration of a single dose of 6-TG, but after 5 daily doses the guanine of the DNA was largely replaced by 6-TG. This indicates that most cells were caused to enter DNA synthesis in the 5-day period.

INTRODUCTION

A series of studies (3–7) in normal and neoplastic tissues of mice indicated that, while 6-TG3 had several metabolic effects, the only one consistently correlating with cell toxicity was the incorporation of 6-TG into DNA in place of the normal guanine component. This finding has implications for the planning of therapeutic regimens, to minimize bone marrow toxicity and to obtain the maximum antitumor response. 6-TG is routinely given p.o. An earlier report (2) indicated that absorption of p.o. dose was incomplete. In order to determine whether incorporation into the bone marrow DNA of humans was related to dosage sequences and further to compare the effects of p.o. and i.v. doses, a study was conducted in a series of patients having either acute leukemia or solid tumors. This was accomplished as part of a Phase I clinical study of 6-TG, in which the optimum dosage was being determined for a 5-day course of daily treatments. Results of the Phase I study will be presented elsewhere.

MATERIALS AND METHODS

The 6-TG used in this study was obtained from Burroughs Wellcome and Co., Research Triangle Park, N. C. It was of 98.6% purity on an anhydrous basis and gave a single spot on each of several paper chromatographic systems (e.g., 5% KH₂PO₄; ethanol:1 M ammonium acetate, 100:6, v/v; isobutyric acid:ammonia:H₂O, 66:1:33, v/v). 6-TG was labeled with radiosulfur by exchange with rhombic sulfur-³⁵S, in refluxing pyridine (3) and was purified until the only detectable radioimpurity (<1%) was apparently rhombic sulfur (no mobility on KH₂PO₄ chromatography). Preparations of 6-TG or 6-TG-³⁵S were stored at −70° to minimize oxidation that otherwise becomes significant over a period of months.

For p.o. administration, 6-TG-³⁵S was provided in gelatin capsules. For i.v. administration, it was converted to a sodium salt by the addition of 1.2 equivalents of NaOH and was dissolved in 0.9% NaCl solution at 10.0 mg/ml. Each dose was injected through a sterile Millipore filter (Swinnex-25 filter units with 0.22-μ pores; Millipore Corp., Bedford, Mass.). Patients given single doses of 6-TG-³⁵S received 75 to 100 μCi. When it was given once a day for 5 days, the solution was held at 2°. The 75 to 100 μCi were then uniformly given in the 5-day course. It was established that significant deterioration did not occur under these conditions for 8 days.

Scintillation counting was carried out, with a Nuclear-Chicago Unilux II system, in plastic vials. Where necessary, quench corrections were determined with an external standard. Aliquots of the 6-TG-³⁵S in use in the individual experiments were counted with each series of samples to obviate a need for decay corrections.

Blood samples were withdrawn into heparinized tubes and promptly refrigerated. Except as noted, they were centrifuged, and plasma was taken off and mixed with an equal volume of 0.4 M PCA. Aliquots of the PCA extracts were used to measure total radioactivity and for chromatography on Whatman No. 3MM papers with carrier compounds added. The systems used for developing the papers were those described above. The chromatograms were air dried; carrier spots were visualized with a UV lamp and cut out for direct scintillation counting. In all cases, total radioactivity and radioactivity in 6-TG were determined. In some of the initial experiments, the 6-TG content was determined on extracts of RBC's, on plasma, and direct plasma samples solubilized with Hyamine.

Urine samples were collected in plastic containers and immersed in ice until analyzed. The volumes were determined, and aliquots were used to determine total radioactivity and 6-TG content. In some cases, chromatography was also used to determine 6-TX, 6-TU, and 6-MeTG. In order to measure these, carrier compounds were added before chromatography, and paper spots containing each component were counted directly in the scintillation system. Systems used and Rₚ values were as follows. With the 5% KH₂PO₄ system, the Rₚ value for 6-TG was 0.25; with isobutyric acid: NH₄OH·H₂O, the...
values for 6-TG, 6-TX, and 6-TU were 0.56, 0.45, and 0.14, respectively; and with ethanol:ammonium acetate, the values for 6-TG, 6-TX, 6-TU, and 6-MeTG were 0.48, 0.35, 0.22, and 0.65, respectively. Inorganic sulfate moved near the solvent front in all of the systems. In some cases, it was necessary to add NH₄OH to urines to solubilize a precipitated radioactive component. Chromatography indicated this component to be 6-TU.

Bone marrows were drawn at the indicated times from the posterior iliac crest into heparinized tubes. They were chilled and centrifuged, and the plasma was discarded. Cold 0.4 M PCA was used to extract 6-TG and acid-soluble nucleotides. DNA and RNA were isolated, and DNA was assayed by a spectrophotometric method, with calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) as a standard (8). The acid-soluble extracts were chromatographed on Whatman No. 3MM papers with 5% KH₂PO₄ (Rₚ for 6-thio-GMP, 6-thio-GDP, and 6-thio-GTP was 0.78).

RESULTS AND DISCUSSION

Thirty-two pharmacological experiments were conducted in 24 patients. Of these, 15 were acute leukemia patients with bone marrow content that was largely neoplastic. Nine patients who had various solid tumors were studied before other treatment and were therefore assumed to have normal bone marrows. In 7 acute leukemia and 7 solid tumor patients given 6-TG i.v. in single doses from 65 to 300 mg/sq m, the plasma half-times were 25 to 240 min, with a median of 80 min. No correlation was observed between half-time and dose, although initial plasma levels of 6-TG did correlate with dose level.

In several experiments, RBC's and plasma were analyzed separately, and plasmas were analyzed both directly and after PCA extraction. A typical result is illustrated in Chart 1. 6-TG levels were very similar for RBC's and plasma, but RBC's contained a somewhat higher level of metabolites. PCA extracts from plasma gave the same levels of total radioactivity as did direct counts on samples solubilized in Hyamine, indicating that extraction was complete. Since extracts were needed for chromatography, PCA extraction was routinely used in subsequent experiments. In Chart 2 are presented the analyses of plasma samples from a solid tumor patient (J. L. K.) given 6-TG-³⁵S: first i.v. and, 22 days later, p.o. Both dosages were at 135 mg/sq m, a total dose of 256 mg. After i.v. dose, the 6-TG level in the plasma dropped rapidly, showed a transient rise at 1 hr (also observed in other cases), and then
Table 1

Incorporation of 6-TG into nucleotides and polynucleotides of bone marrow

Bone marrow samples were used to isolate acid-soluble nucleotides, DNA and RNA, and analyses were made for DNA by a spectrophotometric method, with calf thymus DNA as a standard. All analyses are expressed on the basis of the DNA analysis.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Patient</th>
<th>Regimen</th>
<th>6-TG incorporated ({µg}/{mg DNA})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>J. L. K. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. Marrow analysis at 4 hr.</td>
<td>DNA: 58.2, RNA: 20.6, Mononucleotide: 88</td>
</tr>
<tr>
<td>1</td>
<td>J. L. K. (solid tumor)</td>
<td>135 mg/sq m 6-TG p.o. Marrow analysis at 8 hr. Experiment was 22 days after i.v. dose above.</td>
<td>DNA: 25.8, RNA: 18.7, Mononucleotide: 1.2</td>
</tr>
<tr>
<td>2</td>
<td>V. G. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. Marrow analysis at 4 hr.</td>
<td>DNA: 0.20, RNA: 0.40, Mononucleotide: 1.32</td>
</tr>
<tr>
<td>2</td>
<td>V. G. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. Marrow analysis at 4 hr. Experiment was 9 days after i.v. dose above.</td>
<td>DNA: 1.55, RNA: 2.86, Mononucleotide: 1.15</td>
</tr>
<tr>
<td>3</td>
<td>R. S. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. 4-hr marrow</td>
<td>DNA: 8.3, RNA: 7.75, Mononucleotide: 4.5</td>
</tr>
<tr>
<td>3</td>
<td>R. S. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. 8-hr marrow</td>
<td>DNA: 18.3, RNA: 18.9, Mononucleotide: 4.8</td>
</tr>
<tr>
<td>4</td>
<td>W. F. M. (solid tumor)</td>
<td>135 mg/sq m 6-TG p.o. 4-hr marrow</td>
<td>DNA: 3.84, RNA: 11.9, Mononucleotide: 4.8</td>
</tr>
<tr>
<td>4</td>
<td>W. F. M. (solid tumor)</td>
<td>135 mg/sq m 6-TG p.o. 8-hr marrow</td>
<td>DNA: 2.86, RNA: 6.15, Mononucleotide: 2.3</td>
</tr>
<tr>
<td>5</td>
<td>Sh. (AML)</td>
<td>67.5 mg/sq m 6-TG; single i.v. dose. 4-hr marrow</td>
<td>DNA: 0.70, RNA: 2.23, Mononucleotide: 9.6</td>
</tr>
<tr>
<td>5</td>
<td>Sh. (AML)</td>
<td>80 mg/sq m 6-TG i.v.; 5 daily treatments. Marrow analysis 4 hr after last dose.</td>
<td>DNA: 109, RNA: 508, Mononucleotide: 792</td>
</tr>
<tr>
<td>5</td>
<td>J. A. K. (ALL)</td>
<td>135 mg/sq m 6-TG; single i.v. dose. 4 hr marrow</td>
<td>DNA: 0.59, RNA: 0.45, Mononucleotide: 25.5</td>
</tr>
<tr>
<td>5</td>
<td>J. A. K. (ALL)</td>
<td>175 mg/sq m 6-TG i.v.; 5 daily treatments. Marrow analysis 4 hr after last dose.</td>
<td>DNA: 97, RNA: 359, Mononucleotide: 1820</td>
</tr>
<tr>
<td>6</td>
<td>Con. (AML)</td>
<td>200 mg/sq m 6-TG i.v.; 5 daily treatments. Marrow analysis 4 hr after last dose.</td>
<td>DNA: 117, RNA: 96, Mononucleotide: 970</td>
</tr>
<tr>
<td>6</td>
<td>Con. (AML)</td>
<td>65 mg/sq m 6-TG i.v.; 5 daily treatments, 9 days after arabinosylcytosine course. Marrow analysis 4 hr after last 6-TG dose.</td>
<td>DNA: 59, RNA: 64, Mononucleotide: 600</td>
</tr>
<tr>
<td>7</td>
<td>J. G. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. Marrow analysis at 4 hr.</td>
<td>DNA: 5.28, RNA: 5.68, Mononucleotide: 55.5</td>
</tr>
<tr>
<td>7</td>
<td>N. W. (AML)</td>
<td>135 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 17.5, RNA: 11.8, Mononucleotide: 23.6</td>
</tr>
<tr>
<td>7</td>
<td>J. W. (ALL)</td>
<td>135 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 0.21, RNA: 0.06, Mononucleotide: 2.19</td>
</tr>
<tr>
<td>7</td>
<td>O. F. (AML)</td>
<td>135 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 0.65, RNA: 1.01, Mononucleotide: 8.1</td>
</tr>
<tr>
<td>7</td>
<td>D. W. (AML)</td>
<td>135 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 2.07, RNA: 7.0, Mononucleotide: 29.8</td>
</tr>
<tr>
<td>7</td>
<td>M. D. H. (AML)</td>
<td>135 mg/sq m 6-TG i.v. 9 days after a 5-day arabinosylcytosine course. Marrow at 4 hr.</td>
<td>DNA: 2.26, RNA: 0.24, Mononucleotide: 0.88</td>
</tr>
<tr>
<td>7</td>
<td>R. L. C. (solid tumor)</td>
<td>300 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 55.7, RNA: 56.4, Mononucleotide: 86</td>
</tr>
<tr>
<td>7</td>
<td>J. T. (solid tumor)</td>
<td>300 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 53.8, RNA: 45.6, Mononucleotide: 8.7</td>
</tr>
<tr>
<td>7</td>
<td>T. M. (solid tumor)</td>
<td>135 mg/sq m 6-TG p.o. Marrow at 8 hr.</td>
<td>DNA: 25.8, RNA: 25.8, Mononucleotide: 4.0</td>
</tr>
<tr>
<td>7</td>
<td>Cal. (solid tumor)</td>
<td>135 mg/sq m 6-TG i.v. Marrow at 4 hr.</td>
<td>DNA: 3.53, RNA: 14.3, Mononucleotide: 11.5</td>
</tr>
</tbody>
</table>

remained at a relatively low and declining rate. After the p.o. dose was given, total radioactivity reached a peak at 8 hr and declined slowly. 6-TG levels, after the p.o. dose, remained at a very low and relatively steady level. Chart 3 presents the analyses on urinary excretion by the same patient described in Chart 2. After the i.v. dosage, excretion in 24 hr amounted to 77% of the dose. Some unchanged 6-TG appeared in the urine during the 1st 2 hr. Thereafter, excretion was entirely as metabolites, predominantly 6-TU. After the p.o. dosage, no significant excretion of 6-TG occurred. With a lag of about 2 hr, there was steady excretion, about equally as 6-TU and 6-MeTG, plus a small percentage of sulfate. This confirms an earlier report by Elion et al. (2). The total 24-hr excretion in this case was 46%, higher than in others given p.o. treatment.
In other respects this was a representative case. The 24-hr
excretions for patients given i.v. doses was 41 to 81%. For
patients given p.o. doses, it was 24 to 46%.

Table 1 presents analyses for incorporation of 6-TG-35S
into DNA, RNA, and mononucleotide (a mixture of the
mono-, di-, and triphosphates) of bone marrows. Experiment 1
represents the analyses for the same patient described in
Charts 2 and 3. Incorporation into DNA was approximately
twice as great at 4 hr with an i.v. dose as it was in 8 hr with the
p.o. dose. However, Experiments 3 and 4 illustrate cases where
incorporation into DNA was roughly linear for 8 hr after an
i.v. dose but had ceased by 4 hr after a p.o. dose. Experiment
gives results of 2 tests on the same patient, 1 test while the
marrow was very cellular, and the other, 9 days after the 1st
course of treatment, when recovery was still incomplete. In
the latter case, incorporation was increased to about 7-fold
that of the 1st test. In Experiment 7, a patient (T. M.) given
6-TG p.o. 2 weeks after an earlier course of treatment with
6-TG was still recovering. In this case, a relatively high level of
6-TG in DNA was found. Thus, the growth status of the
marrow is a most significant factor. Analyses on patients
described in Table 1, Experiments 5 and 6, illustrate that,
while the incorporation of 6-TG into DNA in 4 hr after the
initial dose may be very small, the course of 5 daily treatments
must project all or most cells into active growth, since from
one-half to essentially all guanine in the DNA has been
replaced by 6-TG. Complete replacement would be
represented by approximately 110 μg of 6-TG/mg of DNA.

Table 1, Experiment 7, provides analyses on several
additional patients under various conditions. In 2 of the
earliest marrow analyses made, the DNA was degraded with
DNase and phosphodiesterase (3), and the resulting products
were chromatographed on paper. The radioactivity was found
to be present almost entirely in the nucleotide component (90
to 95%), with the balance present in thioguanosine. This
indicated that the incorporation resulted from actual DNA
synthesis. Chromatography of PCA extracts from the bone
marrows seldom showed any free 6-TG. The radioactivity was
present as mononucleotide. The very high incorporations in
DNA resulting from multiple dosage gave high mononucleotide
levels, especially after i.v. dosage. Since guanine nucleotide
pools are very low in mammalian cells, the 6-TG nucleotide
levels found are 1 to 3 orders higher in amount, and it is not
unreasonable for guanine to be totally replaced by 6-TG in
cells making DNA from such precursor pools. Considering the
relatively large nonproliferating fraction of cells indicated by
the incorporation seen with a single dose, the very high
incorporation seen after 5 doses must mean that cell kill is
rapid and that it results in the activation of nonproliferating
cells. Data from recent studies of β-2′-deoxythioguanosine in
synchronized cell cultures indicate that such cells are killed
only in S phase and that progression through the cycle is
unimpeded (1).

Table 2 provides some data on the daily excretion of 6-TG
and products during and after a 5-day course of daily
treatments. The patients had advanced acute leukemia. A
relatively high retention of 6-TG in these patients was
observed. Bone marrow would be expected to be the major
repository of 6-TG not present in the body fluids, since the
gastrointestinal lining and skin rapidly deaminate it, and other
tissues of appreciable mass have very low mitotic rates. Bone
marrow aspirations taken 4 hr after administration of single
doses of 6-TG yielded, in 4- to 5-ml total volume, 1 to 2 mg
DNA. Aspirations of similar size taken 4 hr after a 5-day
course of 6-TG yielded 15 to 40 μg DNA. In some cases, not
analyzed, the yield was less than 15 μg DNA. Thus, this
biological observation confirms that the bone marrow was
responding very rapidly to the 5 treatments and that the cells
present initially were largely gone by 5 days.

ACKNOWLEDGMENTS

We are indebted to Dr. George H. Hitchings for supplying the 6-TG.

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Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Daily dose of 6-TG (mg)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. H. B. (AML)</td>
<td>137</td>
<td>106</td>
<td>85</td>
<td>58</td>
<td>96</td>
<td>109</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>J. A. K. (ALL)</td>
<td>350</td>
<td>263</td>
<td>276</td>
<td>274</td>
<td>251</td>
<td>267</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Sh. (AML)</td>
<td>140</td>
<td>72</td>
<td>112</td>
<td>123</td>
<td>100</td>
<td>79</td>
<td>38</td>
<td>18</td>
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<tr>
<td>Con. (AML)</td>
<td>150</td>
<td>107</td>
<td>114</td>
<td>137</td>
<td>130</td>
<td>111</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>A. V. P. (AML)</td>
<td>74</td>
<td>38</td>
<td>50</td>
<td>57</td>
<td>41</td>
<td>48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Five daily doses given.

b AML, acute myelogenous leukemia.
c ALL, acute lymphocytic leukemia.
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