Phase I Trial of Parenteral 6-Thioguanine Given on 5 Consecutive Days


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ABSTRACT

For almost 30 years, 6-thioguanine (6-TG) has been administered p.o. for treatment of various human cancers, especially leukemias, even though the systemic availability of the drug given p.o. is known to be low and highly variable. Parenterally administered 6-TG has been studied in detail in humans only on a single-day intermittent schedule, although multiple-day intermittent schedules are known to produce maximal cytotoxic effects in several animal species. To develop a multiple-day regimen for parenteral 6-TG therapy, we carried out a dose-seeking and pharmacokinetic study of the drug given i.v. daily for 5 days in patients with various refractory advanced solid tumors. Dose-limiting myelosuppression without other significant toxicity occurred at 55–65 mg/m² daily for 5 days. After i.v. administration at 65 mg/m², the mean peak plasma concentration of 6-TG ranged from 6–10 μM. These concentrations are 8–300 times greater than peak plasma concentrations of 6-TG in plasma reported to occur after p.o. administration at 100 mg/m². We suggest that the antitumor activity of 6-TG be reassessed against human cancers in regimens of i.v. administration on multiple-day intermittent schedules.

INTRODUCTION

The purine analogs 6-TG3 and 6-MP were introduced for treating human cancer approximately 30 years ago (1). 6-MP was shown to be capable of producing complete, albeit transient, remissions in ALL when given p.o. on a chronic schedule (2). This was subsequently verified for 6-TG on the same type of schedule (3, 4). By the mid 1960s, chronic p.o. use of both drugs was well established: 6-MP as a component of maintenance chemotherapy for ALL and 6-TG as a component of combination chemotherapy for the induction and, at times, remission maintenance of ANLL (5). When evaluated in a few patients with ALL, 6-TG on a chronic p.o. schedule was believed to offer no advantage over 6-MP (6). There seems to be a consensus that 6-MP contributes to the efficacy of remission maintenance in ALL but this is not universally accepted. The benefit of 6-TG in the therapy of ANLL is not well established (6).

It is surprising that both thiopurines are given p.o. because plasma concentrations of parent drug are low and highly variable after p.o. administration of 6-MP and 6-TG. As early as 1961, marked variability was reported (7) in the amount of 6-TG excreted in urine and the fraction of parent drug after p.o. administration of 6-MP. They suggested that parenteral regimens of 6-MP should be reevaluated for therapeutic activity in humans (12).

We decided to reevaluate 6-TG rather than 6-MP for clinical use because in vitro (13–15) and in vivo (16–18) evidence suggests that conversion of 6-MP to 6-TG nucleotides is the major determinant of cytotoxic activity of 6-MP as well as of 6-TG. Furthermore, unlike 6-MP, 6-TG is much more cytotoxic when given parenterally on a multiple-day schedule than when given on a single-day intermittent schedule in several species of animals (19). When 6-MP and 6-TG are given parenterally on a multiple-day schedule at doses producing comparable degrees of myelosuppression, 6-MP causes toxicities not encountered with 6-TG, including severe nausea and vomiting, mucositis, and hepatotoxicity in animals (19) and in humans (20–22). For these reasons, we studied the toxicity and therapeutic activity in a dose-seeking study of 6-TG given i.v. daily for 5 days in patients with advanced cancer and normal or near-normal function of major organ systems.

MATERIALS AND METHODS

The study group consisted of 36 patients, 15 men and 21 women aged 25–70 years, with histologically documented advanced solid tumors for which no method of therapy is known to offer hope of cure or of significant palliation. Patients were excluded from the study for the following reasons: leukocyte count, <4,100/μl; platelet count, <130,000/μl; hemoglobin, <10 g/dl; any increase in direct-reacting serum bilirubin; aspartate aminotransferase or alkaline phosphatase, >3 times normal; serum creatinine, >1.5 mg/dl; decreased performance status (requiring >50% of waking hours in bed); parenteral nutrition or hydration required; radiation to >15% of bone marrow within 30 days; radiation to >30% of bone marrow with the equivalent of 15 Gy in 3 fractions. Measurable disease was not required for admission to study. Informed consent as required by institutional and federal regulations was obtained from all patients prior to entry into the study.

6-TG, obtained as the sodium salt from the Division of Cancer Treatment, National Cancer Institute, was dissolved in sterile isotonic saline at a concentration of 15 mg/ml. For every 75 mg of dissolved drug, 0.5 meq of sterile sodium bicarbonate was added to lower the pH from 11.2 to approximately 9.2. Lowering the pH eliminates pain and venous irritation which are otherwise universal during and after administration of the drug.6-TG was given i.v. over 5 min daily for 5 consecutive days. Patients with stability or improvement and whose disease was not progressive were allowed to continue treatment, to a maximum of 60 mg/m² daily.

Blood samples in heparinized tubes containing dithiothreitol at a final concentration of 1 mM were prepared for high-pressure liquid chromatography by the method of Hendrick and Mirkin (23) and judging from their graphs) after p.o. administration than after i.v. administration of the same dose of radiolabeled 6-TG in the same patient.

A decade later, Brox et al. (10) found that, after p.o. administration of 6-TG at 100 mg/m², the plasma concentration varied more than 30-fold among 13 patients with acute myeloblastic leukemia. More recently, Zimm et al. (11, 12) documented in monkeys and humans low and variable bioavailability of parent drug after p.o. administration of 6-MP. They suggested that parenteral regimens of 6-MP should be reevaluated for therapeutic activity in humans (12).

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3 Abbreviations: 6-TG, 6-thioguanine; 6-MP, 6-mercaptopurine; ALL, acute lymphoblastic leukemia; ANLL, acute nonlymphocytic leukemia; ara-C, 1-/3-D-arabinofuranosycytosine.

4 J. S. Kovach, unpublished results.
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assayed by the method of Andrews et al. (24), with substitution of a 5-
μm C18 Ultrasphere column (Beckman Instrument Co., Palo Alto, CA)
and 8-mercaptothiuric acid as an internal standard. Peaks eluting from
the column were detected with a HP1040A high-speed photometric detec-
tor (Hewlett-Packard, Palo Alto, CA) and measured by comparison to
the area of the internal standard. The lower limits of accurate measure-
ment were 10 ng for 6-TG and 6-thiioxanthine (341 nm), 50 ng for 6-
methylthioguanine (310 nm), and 50 ng for 6-thiocycosic acid (347 nm).
Our sensitivity of measurement of 6-TG is comparable to that reported
by a number of investigators using high-pressure liquid chromato-
graphic separation of thiopurines (25-27). We ascribe the greater
sensitivity of our assay compared with that reported by Andrews et al.
(24) to greater column efficiency, judging from their published chro-
matograms. Pharmacokinetic parameters were determined by DRUG-
MODEL, a public procedures facility of the Prophet on-line computer
system (28).

RESULTS

Dose-limiting myelosuppression was achieved at 55–65 mg/m²
daily for 5 days (Table 1). Men seemed to be slightly more
sensitive than women to 6-TG in terms of myelosuppression
although statistical analysis did not support this impression.
There was no evidence of cumulative hematological toxicity,
and there was no other significant toxicity. A few patients had
mild nausea and vomiting on some days of treatment. There
was no suggestion of hepatic, renal, or mucocutaneous toxicity.
In 4 of 18 patients with colon cancer, the disease was stable for
6, 9, 9, and 11 months, respectively. No objectively measurable
therapeutic responses were noted.

The plasma disappearance of 6-TG best fit a one-compartment
model in most instances. The average terminal half-life
of 6-TG elimination from plasma was approximately 20 min
on days 1 and 5 at doses ranging from 30–65 mg/m² (Fig. 1).
Because plasma concentrations of 6-TG were low at all doses
tested and declined rapidly to below the limit of accurate measurement (10 ng/ml), precise and complete determination
of plasma elimination kinetic parameters was not possible.
There were no differences in the rate of disappearance between
men and women (data not shown). Total body clearance ranged
from approximately 2–2.5 liters/min/m². Previous studies in
our laboratory on patients receiving larger doses of 6-TG i.v.
demonstrated that at 700 mg/m² the terminal half-life of 6-TG
plasma elimination was approximately 2.3 h and total body
clearance was 0.32 liter/min/m².2 Konits et al. (29) reported a
terminal half-life for 6-TG of 5.9 h after doses up to 1,200 mg/
m² i.v.

Some 6-TG could be detected in erythrocytes. In 2 patients,
detectable but not reliably measurable amounts of methylthio-
guanine, were present in plasma. 6-Thiouric acid, 6-thioxan-
thine, 6-methylthioxanthine, and the nucleotides of 6-TG were
not detectable in plasma and erythrocytes on day 1 or day 5 up
to 3 h after drug administration.

DISCUSSION

When given p.o. as single agents, 6-TG and 6-MP are known
to be capable of producing complete remissions in some patients
with acute leukemia (4, 5). Both drugs are currently used only
in combination chemotherapy regimens although their efficacy
in these programs is not well defined. In the only randomized
study, p.o. 6-TG added to a standard combination chemother-
apy regimen in patients with ANLL failed to increase the
frequency of induction of complete remission or the duration
of survival compared to the same regimen without 6-TG (30).
Because the systemic availability of 6-TG (10) and of 6-MP
(11, 12, 31) is highly variable after p.o. administration, we
believe that estimates of the therapeutic efficacy of these thio-
purines given p.o. may not reflect their therapeutic potential.
Another factor that may limit the therapeutic success of 6-TG,
at least in ANLL, is the practice of administering 6-TG and
ara-C concomitantly. As early as 1973, LePage and White (32)
demonstrated in mice that parenteral administration of ara-C
immediately before an otherwise fatal parenteral dose of 6-TG
prevents death, presumably by inhibiting DNA biosynthesis
and thereby decreasing the incorporation of 6-TG into DNA.
Given concomitantly with ara-C, 6-TG is believed to be cyto-
toxic only to cells resistant to inhibition of DNA synthesis by
ara-C (32). The same result would be expected when 6-TG is
administered with any agent that inhibits DNA synthesis.

There is considerable evidence that the antitumor activity of
6-TG and of 6-MP depends primarily on metabolism to 6-TG
deoxyribonucleotide and incorporation into DNA (13, 15, 33,
34) although a number of other mechanisms may contribute to
the cytotoxicity of these thiopurines (35-39). Recently, Lennard
et al. (40) reported that, in children with ALL receiving p.o. 6-
MP therapy chronically, the dose of drug correlates with the
amount of 6-TG nucleotides in erythrocytes and with the extent
of delayed (day 14) neutropenia. Among these leukemic chil-
dren, girls were more sensitive than boys to the cytotoxic effect
of 6-MP (18). This observation is intriguing because girls with
ALL are known to have a better prognosis than boys with ALL
have (41). In the present study, doses of 6-TG that produced
limiting myelosuppression were only 1/4th, on a molar basis,
of the i.v. doses of 6-MP needed to produce comparable degrees
of myelosuppression on the same schedule (21).

The present study demonstrates that 6-TG given i.v. daily for

Table 1 Hematological toxicity of 6-TG as a function of dose (initial course)

<table>
<thead>
<tr>
<th>Daily dose (mg/m²)</th>
<th>Median⁴ nadir of cell count (x 10⁹ cells/μl)</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>5.8 (4.1-8.0)</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>3.5 (1.4-10.4)</td>
<td>3</td>
</tr>
<tr>
<td>37.5</td>
<td>2.5 (2.4-5.9)</td>
<td>2</td>
</tr>
<tr>
<td>45</td>
<td>3.7 (2.9-4.7)</td>
<td>3</td>
</tr>
<tr>
<td>50</td>
<td>3.7 (3.7-4.9)</td>
<td>3</td>
</tr>
<tr>
<td>55</td>
<td>3.8 (0.3-5.9)</td>
<td>15</td>
</tr>
<tr>
<td>65</td>
<td>2.2 (2.0-2.6)</td>
<td>6</td>
</tr>
</tbody>
</table>

⁴ Numbers in parentheses, range.

Fig. 1 Plasma 6-TG concentration after rapid i.v. infusion of 55 mg/m² on
day 1 (□) and day 5 (▲), shown as mean ± SD for 9 patients on day 1 and 6
patients on day 5.

5 J. S. Kovach, unpublished data.
5 days consistently produces dose-dependent, reversible, non-cumulative myelosuppression without other toxic effects in patients with advanced solid tumors. At a total dose of 325 mg/m² over 5 days, what we consider to be dose-limiting myelosuppression—leukocyte count, <3,000/µl; platelet count, <100,000/µl—occurred in all patients treated. We (42) and others (29) have demonstrated previously that much larger doses of 6-TG (up to 1,200 mg/m²) given i.v. on a single day result in only minimal cytotoxic effects while producing severe nausea and vomiting. This marked schedule dependence of the cytotoxicity of parenteral 6-TG therapy is identical to that found in animal models 30 years ago by Philips et al. (19).

We believe that repetitive doses of 6-TG produce greater cytotoxic effects than do single large doses of 6-TG because on a multiple-dose schedule more cells are exposed to drug as they enter DNA synthesis. This contention is supported by the fact that, in the present study, doses of 6-TG given daily for 5 days (65 mg/m² per day) that consistently produced limiting myelosuppression were associated with low peak plasma concentrations of drug (mean, 1.5 µg/ml) and rapid plasma elimination (mean terminal half-life, 20 min) (Fig. 1) whereas large doses of 6-TG (800–1200 mg/m²) on a single day, which produce higher peak plasma concentrations (15 µg/ml) and longer terminal half-lives of plasma elimination (350 min), do not consistently produce limiting myelosuppression (29, 42). The long half-life of plasma elimination of 6-TG after a single large dose of drug suggests that metabolism of 6-TG is saturated at high doses, leading to accumulation of drug (and metabolites) (29).

Our clinical results are similar to those reported in a few patients with solid tumors by Krakoff et al. in 1961 (7). They determined the amount of 6-TG given daily at doses from 40 to 100 mg/m² (either i.v. or p.o.) needed to decrease leukocyte count to ≤3000 cells/µl. They found moderate leukopenia in patients receiving 6-TG i.v. at mean total doses of 220 mg/m² (range, 185–290 mg/m²) compared with 275–325 mg/m² in our study. With 6-TG given p.o., however, the total dose required to decrease leukocyte count to ≤3000 cells/µl averaged 2500 mg/m². Of particular importance to the current clinical practice of administering 6-TG p.o. is the fact that the amount of 6-TG required by this route to produce the same degree of myelosuppression varied 6-fold among 8 patients (7). We believe that this variability is due to differences in the delivery of 6-TG into the systemic circulation rather than to differences in the metabolism of 6-TG after it reaches the systemic circulation. This point of view is supported by the data of Brox et al. (10) who documented differences of up to 30-fold in peak plasma concentrations of 6-TG after p.o. administration and by our pharmacokinetic data which demonstrate that i.v. administration of 6-TG is associated with uniform systemic availability of parent drug (Fig. 1).

6-TG has not been studied as extensively as 6-MP primarily because, in early trials of p.o. administration, 6-TG appeared to offer no therapeutic advantage over the clinically established 6-MP (4). Data accumulated over the past 30 years and our present results, however, lead us to conclude that the antitumor activity of 6-TG against various human cancers, including acute leukemias, should be reevaluated when the drug is given i.v. on a multiple-day schedule. As new drug combinations incorporating parenteral administration of 6-TG are developed, it should be remembered that the administration of 6-TG at times when DNA synthesis is inhibited by other agents—as occurs with standard therapy for ANLL (6) and in some experimental aggressive chemotherapy-autologous bone marrow transplantation regimens for advanced lymphomas (43)—will result in decreased incorporation of 6-TG into DNA (32). Administration of 6-TG prior to other components in combination regimens will maximize the primary cytotoxic effect of 6-TG and enhance potentially therapeutically beneficial interactions of 6-TG nucleotides in DNA with other modalities such as X-rays (44) and alkylating agents (45) given after completion of 6-TG therapy.

REFERENCES

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