A Pediatric Phase I Trial and Pharmacokinetic Study of Thioguanine Administered by Continuous i.v. Infusion

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ABSTRACT

Although mercaptopurine is the thiopurine antimetabolite predominantly used in the treatment of childhood acute lymphoblastic leukemia (ALL), thioguanine (TG) is more potent than mercaptopurine in in vitro cytotoxicity studies in human leukemic cell lines and leukemic cells from patients with ALL. We conducted a pediatric Phase I trial of TG administered as a continuous i.v. infusion (CIV). A pharmacokinetically guided dose escalation was performed to define the dose rate of TG required to achieve a steady-state plasma concentration (C_s) exceeding the target concentration of 1 μM, and then the maximum tolerated duration of infusion of TG at this dose rate was defined. Eighteen patients (median age, 18 years; range, 4–25 years) with refractory malignancies (16 solid tumors and 2 ALL) were enrolled in this study. The starting dose rate of 10 mg/m²/h administered for 24 h achieved an average C_s of 0.9 μM (range, 0.7–1.2 μM). Therefore, the dose rate was escalated to 20 mg/m²/h, which achieved an average C_s of 4.1 μM (range, 1.0–8.3 μM). This disproportionate increase in the C_s of TG suggested a capacity-limited (saturable) elimination process, and a pharmacokinetic model incorporating two compartments with capacity-limited elimination from the central compartment was developed to describe the disposition of TG. The TG clearances (derived from model parameters) at the 10- and 20-mg/m²/h dose rates were 987 and 608 ml/min/m², respectively. Dose-limiting myelosuppression (absolute granulocyte count < 500/mm³ and platelet count < 25,000/mm³) was observed in two of three patients treated with a dose rate of 20 mg/m²/h administered for 36 h. Administration of CIV of TG at 20 mg/m²/h for 24 h was well tolerated in nine patients. Nonhematological toxicities included nonneutropenic infections and mild, reversible changes in hepatic function tests. The recommended dose rate and duration for CIV of TG is 20 mg/m²/h for 24 h.

INTRODUCTION

The thiopurine antimetabolites have been in clinical use for more than 40 years, primarily in the treatment of acute leukemias. MP³ is an important component of maintenance therapy for children with ALL, whereas TG is used primarily in the treatment of acute nonlymphoblastic leukemia (1). The preference for MP over TG in the treatment of ALL is historically based and does not have a strong pharmacological rationale. In the limited early clinical trials, the antileukemic activity of MP and TG seemed similar (2).

There are several experimental observations that suggest that TG has a stronger pharmacological rationale than MP for the treatment of ALL. Thiopurines are prodrugs and require intracellular conversion to TGNs that exert their cytotoxic effect primarily through incorporation into DNA. The intracellular conversion of TG to TGMP is more direct, requiring only one enzymatic step (catalyzed by hypoxanthine-guanine phosphoribosyltransferase), whereas the conversion of MP to TGMP requires three enzymatic steps (3, 4). TGMP is subsequently phosphorylated by kinases to TG triphosphate, the active intracellular metabolite, which is subsequently incorporated into DNA. In cytotoxicity studies performed in vitro with human leukemic cell lines and leukemic cells from patients with ALL, TG was 10-fold more potent and less schedule-dependent than MP (5). Finally, in a small comparative study in children with relapsed ALL, oral TG was better tolerated and produced, on average, 4-fold higher erythrocyte TGN levels than oral MP (6). Erythrocyte TGN levels are a surrogate marker of thiopurine activation and are correlated with toxicity and efficacy of oral MP therapy in ALL (7).

Thiopurines are usually administered p.o., but this route of administration has several limitations. The bioavailability of MP is low (approximately 16%), and the resulting plasma concentrations exhibit considerable interpatient and intrapatient variability (8). In addition, plasma concentrations of MP in most patients are below concentrations required to produce cytotoxicity in vitro. Absorption of oral TG also seems to be limited and variable (9). These pharmacokinetic limitations of oral administration can be circumvented by administering thiopurines i.v. A CIV schedule for MP (10–12) is well tolerated at a dose rate of 50 mg/m²/h for up to 36 h, and plasma concentrations at this
dose rate exceed the threshold concentration and duration of exposure required to produce cytotoxicity in vitro. Reversible, dose-limiting toxicities from CIV of MP for 48 h included elevation of serum transaminases, myelosuppression, and mucositis. CIV of MP has now been incorporated into several childhood ALL treatment regimens (13-15).

On the basis of our prior experience with CIV of MP and the strong pharmacological rationale for the use of TG in ALL, we designed and conducted a pediatric Phase I trial and pharmacokinetic study of CIV of TG. In the first stage of this trial, a pharmacokinetically guided dose escalation was performed to define the dose rate of TG required to achieve a target C\text{\textsubscript{\text{a}}} exceeding 1 \mu M, the in vitro concentration that produced maximum cytotoxicity in human leukemic cell lines (5). In the second stage, the maximum tolerated duration of infusion of TG administered at this dose rate was defined.

**MATERIALS AND METHODS**

**Patient Eligibility.** Patients were eligible for participation in this study if they were ≤25 years of age and had histologically confirmed cancer refractory to standard therapy. Patients must have recovered from the toxic effects of prior therapy and had adequate hepatic function (bilirubin and alanine aminotransferase < 2 \times the upper limit of normal) and renal function (creatinine < 1.5 mg%). To be evaluable for hematological toxicity, patients were required to have an AGC exceeding 1,500 granulocytes/mm\textsuperscript{3}, a hemoglobin exceeding 8.0 gm\% and an Ht exceeding 100,000 platelets/mm\textsuperscript{3}. Written informed consent was obtained before entry into this study. Laboratory assessments of hematological, renal, and hepatic functions were monitored closely throughout the observation period.

**Study Design and Drug Administration.** This Phase I trial was conducted in two stages. The initial stage was designed to determine the dose rate necessary to achieve an average TG C\text{\textsubscript{\text{a}}} of >1 \mu M. The starting dose rate of 10 mg/m\textsuperscript{2}/h for 24 h (total dose, 240 mg/m\textsuperscript{2}) was based on preclinical and adult pharmacokinetic studies (5, 16). The C\text{\textsubscript{\text{a}}} in two of three patients treated at this starting dose rate was below the target concentration of 1 \mu M, providing the basis for a dose rate escalation to 20 mg/m\textsuperscript{2}/h. The dose rate that achieved the target plasma TG concentration was defined as the recommended dose rate. In the second stage of the trial, the duration of infusion was escalated in 12-h increments at the recommended dose rate. This approach allowed for escalation in total dose administered similar to that which would be achieved using a modified Fibonacci sequence, with infusion duration being the escalated parameter. The maximum duration of infusion was defined as the longest infusion duration at which less than two of a cohort of three to six patients experienced a DLT. Toxicities were graded according to the National Cancer Institute/Cancer Therapy Evaluation Program Common Toxicity Criteria, and DLT was defined in this trial as Grade IV neutropenia or thrombocytopenia or any Grade III or IV nonhematological toxicity with the following exceptions: (a) Grade III nausea and vomiting; (b) Grade III hepatotoxicity that returns to Grade I; or (c) Grade III fever. Patients had serum chemistries and complete blood counts performed twice weekly, and complete blood counts were performed a minimum of every other day during periods of neutropenia. A minimum of three patients were treated at each dose level, at least two of whom were evaluable for hematological toxicity. Treatment cycles were repeated every 21 days.

TG was supplied by the National Cancer Institute as a sterile, lyophilized powder in 10-ml vials. Sufficient sodium hydroxide was added to yield the sodium salt of TG. Each 75-mg vial was reconstituted with 5 ml of 0.9% sodium chloride. This was further diluted with 5% dextrose to yield a final TG concentration between 1 and 5 mg/ml. For drug that was to be administered via a peripheral venous catheter, 1 meq of sodium bicarbonate was added for each 150 mg of TG.

**Pharmacokinetics.** Three to 5 ml of whole blood were collected in heparinized tubes 3 and 6 h after the start of the infusion, immediately before termination of the infusion, and at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 12.0 h after completion of the CIV of TG. Samples were immediately placed on ice, and the plasma was rapidly separated by centrifugation and stored at -20°C until assayed.

All chemicals, unless otherwise stated, were obtained from Sigma Chemical Company (St. Louis, MO). Plasma TG concentration was measured with a modification of a previously described reverse-phase HPLC method (10). TG was initially extracted from plasma using Waters C-18 Sep-Pak solid-phase extraction cartridges (Milford, MA), which had previously been primed with 2 ml of methanol and washed with 5 ml of 0.2% glacial acetic acid. Plasma samples (0.5 ml) were spiked with 10 \mu l of 0.5 M DTT and 10 \mu l of 200 \mu M MP riboside (internal standard) and loaded onto the cartridges. The cartridges were rinsed with 3 ml of 0.2% glacial acetic acid, and the samples were then eluted with 2 ml of methanol. Samples were evaporated to dryness under a gentle stream of nitrogen, and reconstituted with 100 \mu l of mobile phase, which was injected onto the HPLC system. The HPLC system included a Waters model 510 pump, a Waters WISP 712 automated sample injector, and a Beckman Ultrasphere ODS 4.6-mm × 25-cm column (5-\mu m particle size; Beckman Instruments, San Ramon, CA). Mobile phase consisted of 0.2% acetic acid buffer and 2.5% acetonitrile at a flow rate of 1.4 ml/min. The eluant was monitored with a Waters 490 multiwavelength detector at wavelengths of 320 and 340 nm. Retention times under these conditions were approximately 5.7 min for TG and 11.2 min for MP riboside.

A two-compartment pharmacokinetic model with capacity-limited (Michaelis-Menten) elimination (Fig. 1) was fit simultaneously to the plasma concentration-time data from patients treated for 24 h at both the 10- and 20-mg/m\textsuperscript{2}/h dose rates using the MLAB program. (17). The Cl of TG was calculated at both infusion rates using the formula

\[ Cl = \frac{V_e \cdot V_m}{K_m + C_{in}} \]

in which \( V_e \) is the volume of the central compartment, \( V_m \) is the maximum rate, and \( K_m \) is the Michaelis-Menten constant (see Fig. 1).

**RESULTS**

**Patient Characteristics.** Eighteen patients were entered onto this study, 11 of whom were evaluable for hematological toxicity. Patients not evaluable for hematological toxicity in-
Fig. 1  Two-compartment pharmacokinetic model with capacity-limited elimination. $C_c$, concentration of TG in the central compartment; $V_c$, the volume of the central compartment; $K_m$, the TG infusion rate; $V_m$, the maximum rate; $K_m$, the Michaelis-Menten constant describing the capacity-limited elimination; $K_p$, the rate constants for the transfer of TG to and from the peripheral compartment; $X_p$, the amount of TG in the peripheral compartment.

$$\frac{dC_c}{dt} = \frac{k_0}{V_c} - \frac{V_m \cdot C_c}{(k_m + C_c)} - k_{cp} \cdot C_c + \frac{k_{pc} \cdot X_p}{V_c}$$

$$\frac{dX_p}{dt} = k_{cp} \cdot V_c \cdot C_c - k_{pc} \cdot X_p$$

Fig. 2  Plasma concentration-time profiles for patients treated with TG as a 24-h continuous infusion. Points, the mean concentration at the 10 (Δ)- and 20 (●)-mg/m²/h dose rates; bars, SDs. Lines, the model-predicted plasma concentration at each dose rate.

Table 1  Patient characteristics

| Age (yrs) | 18 (4–25 yrs) |
| Sex | Male/female 12/6 |
| Prior treatment | Chemotherapy 18 |
| Radiation therapy | 8 |
| Diagnosis | Primitive neuroectodermal tumor/ 8 |
| Ewing's sarcoma | 3 |
| Rhabdomyosarcoma | 2 |
| ALL | 1 |
| Osteosarcoma | 2 |
| Adrenocortical carcinoma | 1 |

included one patient each with insufficient laboratory data or concurrent spinal radiation therapy and two patients each with ALL or prior extensive pelvic radiation therapy. One patient was not evaluable for any toxicity (including hematological) or response because technical problems with the infusion pump led to inaccurate dosing of TG. This patient had Grade II thrombocytopenia but did not experience any other toxicities. Patient characteristics are summarized in Table 1.

Pharmacokinetics. Four patients treated at the 10-mg/m²/h dose rate for 24 h and 13 patients treated at the 20-mg/m²/h dose rate (9 patients for 24 h, and 4 patients for 36 h) had pharmacokinetic sampling performed during the first cycle of administration. For the pharmacokinetically guided dose rate escalation, $C_{ss}$ was defined as the plasma concentration measured during the last hour of the infusion. The $C_{ss}$ of TG was measured in three of four patients treated at the 10-mg/m²/h dose rate, and two of three patients had a $C_{ss}$ value below the 1-μM target concentration ($C_{ss}$ values were 0.7, 0.9, and 1.2 μM). Therefore, the dose rate was escalated to 20 mg/m²/h, which achieved the target $C_{ss}$ (mean $C_{ss}$ of 41 ± 4.1 μM, range 1.0–8.3 μM). This 4-fold increase in the $C_{ss}$ of TG resulting from the 2-fold increase in the infusion rate suggested that TG was eliminated by a capacity-limited (saturable) process. In addition to TG, an apparent metabolite was detected on HPLC that eluted immediately after TG at 6.5 min.

A pharmacokinetic model incorporating two compartments with capacity-limited elimination from the central compartment (Fig. 1) was fit to the concentration-time data from all patients treated at both the 10- and 20-mg/m²/h dose rates as a 24-h infusion, simultaneously. The model parameters derived from the MLAB fit were $V_c = 56 ± 13$ liters/m²; $V_m = 3.7 ± 1$ μM·h⁻¹, $K_m = 2.5 ± 0.5$ μM, $K_{cp} = 0.42 ± 0.23$ h⁻¹, and $K_{pc} = 0.29 ± 0.08$ h⁻¹. The area under the curve of TG (derived from model parameters) at the 10- and 20-mg/m²/h dose rates were 987 and 608 ml/min/m², respectively. The plasma TG concentration-time profiles for patients treated at the 10- and 20-mg/m²/h dose rates for 24 h are shown in Fig. 2.

Toxicity. None of the four patients (two patients were evaluable for hematological toxicity) treated at the 10-mg/m²/h dose rate experienced DLT. Administration of the CIV of TG at the recommended dose rate of 20 mg/m²/h for 24 h was also well tolerated. None of the nine patients, including the six patients evaluable for hematological toxicity, experienced DLT at this dose level. However, at 20 mg/m²/h for 36 h, dose-limiting myelosuppression (AGC < 500 granulocytes/mm³ or Plt < 25,000 platelets/mm³) was observed in two of three patients evaluable for hematological toxicity, with a median AGC nadir of 490 granulocytes/mm³ and a median platelet nadir of 48,000 platelets/mm³ (Table 2). Therefore, the maximum tolerated duration of infusion at 20 mg/m²/h was 24 h. Nonhematological non-DLTs included two patients with nonneutropenic infections and two patients with reversible changes in hepatic function tests. In the three patients who received more than one course of therapy (one patient at 10 mg/m²/h and two...
patients at 20 mg/m²/h for 24 h), there was no evidence of cumulative toxicity.

**Responses.** Of the 18 patients treated on this study, 17 were evaluable for response. Three patients had stable disease [one patient each with rhabdomyosarcoma (duration, 6 weeks), primitive neuroectodermal tumor (8 weeks), and undifferentiated sarcoma (20 weeks)]. There were no complete or partial responses.

**DISCUSSION**

TG administered by CIV is well tolerated at an infusion rate (20 mg/m²/h) and infusion duration (24 h) that achieve plasma TG concentrations in excess of the drug exposure required to produce cytotoxicity in vitro against human leukemia cell lines (5). Myelosuppression was the DLT, and other non-hematological toxicities were mild. MP administered by CIV at a dose rate of 50 mg/m²/h achieves plasma concentrations of 6.9 ± 0.4 μM, and infusion durations of up to 36 h were tolerable (10). However, in addition to myelosuppression, mucositis and hepatotoxicity were prominent with CIV of MP, and, as a result, shorter infusion durations (6–24 h) of CIV of MP have been used in frontline ALL regimens. Both TG and MP are schedule-dependent drugs, demonstrating greater cytotoxicity with increasing durations of exposure. On the basis of preclinical cytotoxicity studies in human leukemic cell lines, longer durations of exposures were required with MP to achieve a cytotoxicity equivalent to that of TG. Exposure to MP for more than 12 h was required to achieve a greater than 50% cell kill, whereas for TG, greater than 50% cell kill occurred with exposures as short as 4 h (5).

The primary mechanism of cytotoxicity for both MP and TG is via incorporation into DNA after conversion to TGNs. For MP, an additional potential mechanism of cytotoxicity is via inhibition of de novo purine synthesis by the MP metabolite TEMP. Despite the fact that TEMP is not formed during TG catabolism, several studies have demonstrated that TG is more cytotoxic than MP (5, 18–20). This suggests that although TEMP may contribute to the cytotoxicity of MP, the greater incorporation of TGNs that occurs with TG results in its superior cytotoxicity.

A number of clinical trials evaluating i.v. administration of TG have been performed in adults (16, 21–24). Myelosuppression was the primary toxicity observed in these trials, regardless of the schedule. The most common schedule used in adults was a daily i.v. bolus dose of 35–55 mg/m²/day for 5 consecutive days. At the 55 mg/m² bolus dose, the mean peak plasma TG concentration was 5–10 μM, but it rapidly declined to less than 1 μM within approximately 1 h (16). Other schedules of CIV of TG include a trial of 5 mg/m²/h for 7 days in adults with acute leukemia (24) and 1.5 mg/m²/h for 5 days in adults with head and neck cancer (23). The higher-dose 7-day infusion, which would be expected to achieve a Cₚₑ of approximately 0.5 μM based on our model, produced two complete and one partial remissions. The DLT was mucositis, but patients treated on this trial would not have been evaluable for hematological toxicity because of bone marrow involvement with leukemia.

Similar to prior studies of i.v. administration of TG, an unknown metabolite was detected in patient plasma (22). Identification of this metabolite is currently ongoing. Our pharmacokinetic studies revealed a 4-fold increase in TG Cₚₑ with a doubling of the dose rate. Dose-dependent Cİ of TG was also suggested by comparison of adult Phase I pharmacokinetic trials that administered TG as an i.v. bolus (16, 22). The Cİ of TG at doses of 30–65 mg/m² exceeded 2000 ml/min/m² (22), but at doses of up to 1200 mg/m², the Cİ was 320 ml/min/m² (16). On the basis of these observations and data presented here, escalation of the TG dose must be undertaken with caution, because the increase in plasma drug concentration may be disproportionately larger than the increase in the dose. The pharmacokinetic model presented in this report may be useful in predicting plasma TG concentration at different doses, but the model parameters were derived from a narrow dosage range and may require additional adjustments.

On the basis of the preclinical pharmacology and its mechanism of action, TG may offer an advantage over MP in the treatment of childhood ALL. The CIV of TG schedule of administration studied here, which will minimize interpatient variability and maintain cytotoxic concentrations for 24 h with minimal toxicity, is currently being evaluated as a component of multiagent chemotherapy, including oral TG, in children with lower-risk ALL. In addition to evaluating the tolerability of repetitive doses of i.v. administered TG, the trial is examining the tolerability of i.v. administration of TG when combined with oral methotrexate.

**REFERENCES**


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