Cancer Chemother Pharmacol (1981) 6:35–38

Cancer Chemotherapy and Pharmacology

Clinical Pharmacology of Oral Thioguanine in Acute Myelogenous Leukemia

L. W. Brox, L. Birkett, and A. Belch

McEachern Laboratory and Cross Cancer Institute, University of Alberta, Edmonton, T6G 2H7, Canada

Summary. *Although thioguanine has been in clinical use for over 20 years, few data are yet available on the clinical pharmacology of thioguanine administered orally. We have studied the plasma thioguanine levels in acute myelogenous leukemia patients during remission induction (daunomycin 60 mg/m^2 on day 1, arabinosylcytosine 200 mg/m^2 · day for 7 days by infusion, thioguanine 100 mg/m^2 PO every 12 h for 7 days) and remission maintenance (arabinosylcytosine 200 mg/m^2 · day for 4 days by infusion, thioguanine 100 mg/m^2 PO every 12 h for 4 days). Hourly blood samples were taken after thioguanine administration, and plasma thioguanine levels were measured by high-performance liquid chromatography with an anion-exchange column. Prior to the chromatography the thioguanine was oxidized by alkaline potassium permanganate to the corresponding 6-sulfonate, which was monitored by means of fluorescence detection. Peak plasma levels of thioguanine were observed 2–4 h after administration and varied from 0.03–0.94 μM. Plasma levels of thioguanine were markedly lower in patients with severe nausea and emesis. Food intake at the same time as thioguanine administration also tended to lower plasma drug levels. The 30-fold range in thioguanine plasma levels observed in this study suggests that intermittent IV administration may provide a better means of standardizing the dosage of thioguanine.*

Introduction

Thioguanine was introduced into clinical use in the mid-1950s and its predominant use has been in the treatment of hematological malignancies. Despite the long clinical experience with thioguanine only one pharmacology study in man has been reported, and this study concentrated on the extent of incorporation of thioguanine after IV administration into intracellular acid-soluble and acid-insoluble constituents [5]. In this study LePage and Whitecar did present data recorded in one patient showing that IV administration of thioguanine resulted in significantly higher drug plasma levels than resulted from the same oral dose.

In this study we have employed a high-performance liquid chromatography method to examine the plasma levels of thioguanine administered orally to patients with acute myelogenous leukemia either during remission induction or during maintenance therapy.

Reprint requests should be addressed to: L. W. Brox

Materials and Methods

The study population consisted of adults with acute myeloblastic leukemia (AML) treated in our institute. The remission induction chemotherapy consisted of daunomycin (60 mg/m^2) IV on day 1, cytosine arabinoside (200 mg/m^2 · day) by continuous IV infusion for 7 days, and oral thioguanine (100 mg/m^2) every 12 h for 7 days. Remission maintenance chemotherapy was given every 6 weeks with a 4-day course of cytosine arabinoside and thioguanine as described above.

Only the morning administration of thioguanine was evaluated in this study. The drug was given while the patient was fasting or 5–15 min before a light breakfast of juice, egg, and toast. Heparinized blood samples (5 ml) were collected at hourly intervals through an indwelling IV cannula. The blood samples were immediately centrifuged and perchloric acid was added to the plasma to a final concentration of 0.4 *M*. After 30 min at 0° C the extracts were centrifuged and neutralized to pH 6.0–6.5 with KOH; the potassium perchlorate was then removed by centrifugation and the extracts stored at −20° C.

Thioguanine and thioguanine nucleotides were measured by high-performance liquid chromatography as described by Tidd and Dedhar [8]. Chromatography of the oxidized thioguanine samples was carried out isocratically at room temperature on a Whatman Partisil-10SAX anion exchange column (25 cm × 4.6 mm) with 0.015 *M* potassium phosphate, pH 4.0, at a flow rate of 1.3 ml/min. Column effluent was monitored for fluorescence by means of a Schoeffel FS-970 detector with an excitation wavelength of 330 nm, an auxiliary Corning 7–54 prefilter, and a 389 nm cut-off emission

0344-5704/81/0006/0035/$ 01.00

filter. A full-scale recorder deflection would correspond to approximately 10 pmol oxidized thioguanine at the detector sensitivity used in these studies. Peak areas were determined with a digital integrator and related to a standard curve obtained by oxidizing known amounts of thioguanine. Sample volumes injected into the column were adjusted so that at least 2 pmol oxidized thioguanine was present. Oxidation of 6-thioxanthine gives rise to a fluorescent product with approximately one-tenth the intensity displayed by thioguanine with the indicated excitation and emission wavelengths. The 6-methylated thiopurines did not produce fluorescent derivatives, and such metabolites were not detected by UV absorption in plasma extracts. Urine samples were not studied for thioguanine metabolites.

Bone marrow aspiration was done on day 3 of the 4-day remission maintenance chemotherapy program, within 1 h after the peak plasma thioguanine level was reached. The marrow sample was promptly centrifuged and the buffy coat was collected and washed once with phosphate-buffered saline. A perchloric acid extract was made and the thioguanine nucleotides were measured as above.

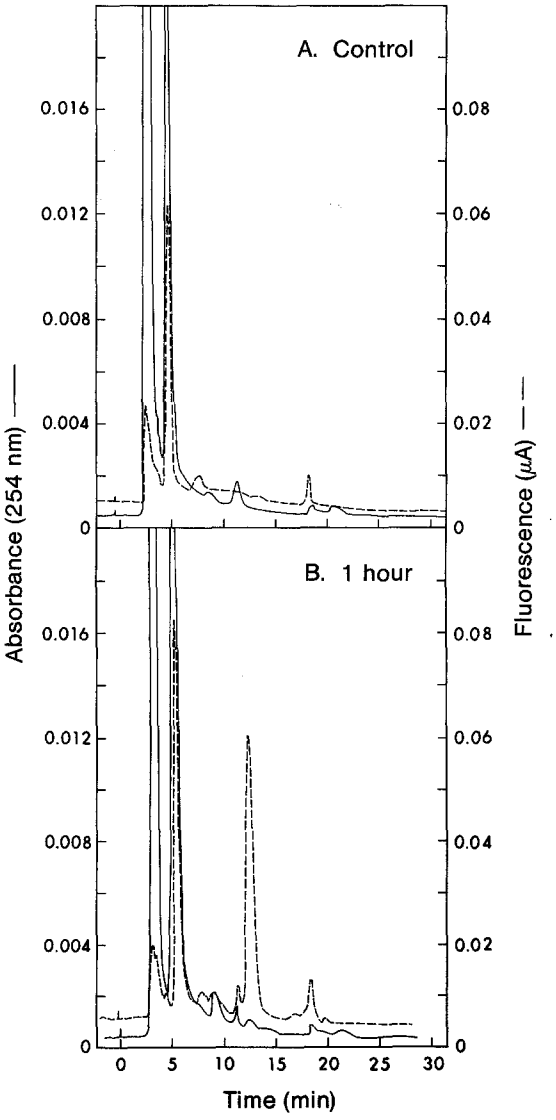

Fig. 1 A and B. Anion exchange chromatography of perchloric acid extracts of plasma samples taken either before (**A**) or 1 h after administration of thioguanine at 100 mg/m^2 PO (**B**)

Results

A typical chromatogram of a permanganate-oxidized plasma extract before and 1 h after an oral dose of thioguanine is shown in Fig. 1.

At the detector sensitivity used in this study, we saw no fluorescent peak corresponding to oxidized thioxanthine in any of the samples analyzed. The peak plasma thioguanine levels from 25 experiments with 13 patients are shown in Table 1. Multiple values for a single patient correspond to different chemotherapy cycles of thioguanine administration. Five of the 25 studies were conducted during remission induction therapy. The peak plasma levels ranged from 0.03–0.84 μ*M* with the time to peak level being about 4 h when food was given or about 1.5 h with no food. This trend is not statistically significant, however, since when evaluated by a two-tailed Student's *t*-test, *P* is between 0.05 and 0.1. In four of five instances higher peak levels were observed when the drug was given to a fasting patient. An example of the effect of food on plasma thioguanine concentration is shown in Fig. 2, where on two successive mornings oral thioguanine was administered either immediately before or without breakfast. In this

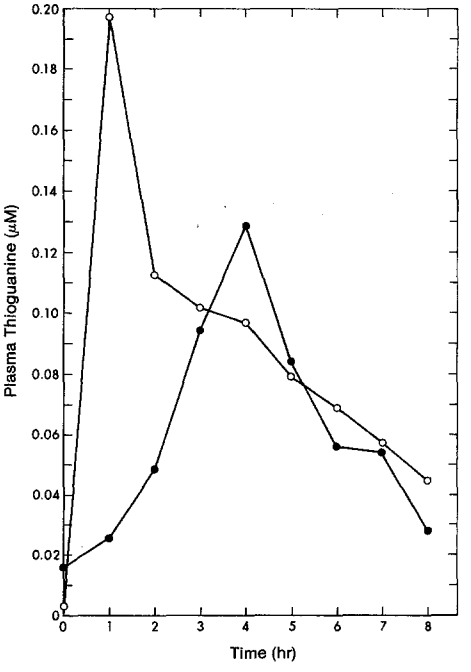

Fig. 2. Plasma thioguanine concentrations as a function of time after an oral dose of 100 mg/m^2. Thioguanine was given either before breakfast (●), or without breakfast (○)

instance the patient had no nausea or emesis on either day.

The 12-h carry-over plasma level of thioguanine was very low except in one instance, where a prolonged time of drug absorption was observed (Table 1). The lowest plasma levels of thioguanine were observed in patients who were severely nauseated and who vomited 60–90 min after drug administration. There was no relationship in this study between the peak plasma levels obtained and the number of cycles of chemotherapy the patient had undergone, or between drug level and white blood cell count.

The plasma disappearance curves for thioguanine were generally similar to that shown in Fig. 2. There was a rapid decrease after the peak level was attained, and this was followed by a gradual decrease. The average plasma thioguanine half-life for the inital rapid decrease was 110 min, with a range between 45 and 240 min. In four cases the plasma thioguanine level remained at the peak level (± 20%) for 2–4 h before the rapid decline occurred. This latter type of plasma disappearance curve appeared to be unrelated to food intake.

Table 1. Concentrations of thioguanine in plasma

Patient	Peak concentration		12-h concentration	Comments
	μM	Time (h)	(μM)	
1. F. A.	0.33	2	–[a]	[b, d]
	0.94	4	–	[b, d]
	0.57	2	–	[c, d]
	0.32	3	–	[c, d]
2. K. E.	0.36	2	0.07	[c, d]
	0.32	1	–	[c, e]
3. J. H.	0.13	4	0.02	[c, d]
	0.20	1	–	[c, e]
	0.27	2	–	[c, e]
4. J. L.	0.09	5	0.05	[c, d, f]
	0.03	3	0.01	[c, d, f]
5. H. L.	0.08	5	–	[c, d, f]
6. J. M.	0.54	6	–	[b, d]
	0.19	2	< 0.01	[b, d]
7. G. P.	0.13	4	< 0.01	[c, d]
	0.08	2	–	[c, d]
	0.24	2	–	[c, e]
8. J. T.	0.09	1	–	[b, d, f]
9. J. W.	0.09	4	0.02	[c, d]
10. G. C.	0.04	9	0.03	[c, d]
	0.33	4	–	[c, e]
11. A. S.	0.06	5	–	[c, d]
12. C. M.	0.57	9	0.33	[c, d]
13. B. B.	0.39	4	–	[c, d]
	0.04	2	–	[c, d, f]

Thioguanine was administered at 100 mg/m² PO every 12 h
[a] Data not available
[b] Remission induction therapy
[c] Maintenance therapy
[d] Thioguanine given before breakfast
[e] No breakfast given
[f] Patient had nausea and emesis

Discussion

It is generally believed that the cytotoxic effects of thioguanine are related to its incorporation into the cellular DNA [4]. Thioguanine must be anabolized to the nucleotide level before such incorporation may occur, and this conversion to the active form is dependent upon the concentration and time of exposure to the drug [1, 5]. Thioguanine may also be catabolized to inactive metabolites. Another important consideration is the plasma level of hypoxanthine and/or guanine. These compounds would reduce the amount of intracellular thioguanine nucleotides by competing with thioguanine in the hypoxanthine-guanine phosphoribosyltransferase-catalyzed reaction [1, 7]. For these reasons, the plasma thioguanine concentration versus time relationship is an important factor in the action of this drug.

In the vast majority of cases thioguanine is still administered orally, despite the work of LePage and Whitecar showing that IV administration of thioguanine resulted in more extensive incorporation into DNA and the work of Elion et al. showing incomplete thioguanine absorption after oral doses [2, 5]. Examination of recent clinical protocols for hematological malignancies employing thioguanine reveals that the most common dosage of this agent is 100 mg/m² every 12 h, during induction or maintenance therapy. The historical selection of this dosage is unclear, as we are not aware of any data describing the myelosuppressive effect of such an oral dosage. This is due in part to the fact that thioguanine has not been used extensively as a single agent. The data presented in this study show that the peak plasma levels of thioguanine resulting from this conventional oral dosage of 100 mg/m² every 12 h varied over a 30-fold range from 0.03–0.94 μM. Furthermore, when food was given with the drug there was a trend towards lower thioguanine peak plasma levels and the time taken to reach these levels was longer.

There is relatively little information available on the plasma levels of thioguanine required for effective bone marrow cell kill. From the study of LePage and Whitecar it is seen that the plasma levels of thioguanine in two patients receiving 135 mg/m²

thioguanine IV was in excess of 15 μ*M* for 5–10 h [5]. In cell culture studies with the RPMI-6410 human lymphoblastoid cell line we have observed that a continuous thioguanine exposure of 0.5 μ*M* is required for complete inhibition of cell growth and cell lysis. In only 4 of the 25 thioguanine courses listed in Table 1 does the peak plasma level of thioguanine exceed 0.5 μ*M*. The intracellular level of 6-thio-GTP in the afore-mentioned study of the RPMI-6410 cells reached approximately 50 nmoles per 10^9 cells. LePage and Whitecar have reported even higher levels of thioguanine nucleotides in the marrow of patients receiving thioguanine IV. Nonetheless, in five patients on this study from whom marrow samples were available shortly after the peak plasma levels were reached we could not detect intracellular thioguanine nucleotides (less than 0.5 nmoles per 10^9 cells). These five patients were all receiving maintenance therapy for AML, and the marrow samples were obtained on day three of therapy. Peak plasma levels of thioguanine were less than 0.27 μ*M* in all cases.

This study illustrates the erratic oral absorption of thioguanine and the frequently low peak plasma levels. In the light of this data, the extent of the contribution made by oral thioguanine in this combination chemotherapy program for AML is questionable. In a recent large clinical trial of AML the addition of oral thioguanine, 100 mg/m^2 daily, to a regimen of daunorubicin, cytosine arabinoside, and prednisone did not increase the remission rate over that obtained with the last three drugs [3]. We suggest that the IV formulation of thioguanine (NSC-752) may be superior to the same drug given PO. At this time several phase II trials are under way, sponsored by the U.S. National Cancer Institute, to assess this question.

Acknowledgements. This work was supported by the National Cancer Institute of Canada and by an equipment grant from Burroughs Wellcome Ltd., Lachine, Quebec.

References

1. Brockman RW (1963) Mechanisms of resistance to anticancer agents. Adv Cancer Res 7: 129
2. Elion GB, Callahan SW, Hitchings GH, Rundles RW (1960) The metabolism of 2-amino-6-[(1-methyl-4-nitro-5-imidazolyl thio] purine in man. Cancer Chemother Rep 8: 47
3. Finnish Leukemia Group (1979) The effect of thioguanine on a combination of daunorubicine, cytarabine and prednisone in the treatment of acute leukemia in adults. Scand J Haematol 23: 124
4. LePage GA (1963) Basic biochemical effects and mechanism of action of 6-thioguanine. Caner Res 23: 1202
5. LePage GA, Whitecar JP Jr (1971) Pharmacology of 6-thioguanine in man. Cancer Res 31: 1627
6. Moore EC, LePage GA (1958) The metabolism of 6-thioguanine in normal and neoplastic tissues. Cancer Res 18: 1075
7. Sartorelli AC, LePage GA (1958) Metabolic effects of 6-thioguanine. II. Biosynthesis of nucleic acid purines in vivo and in vitro. Cancer Res 18: 1329
8. Tidd DM, Dedhar S (1978) Specific and sensitive combined high-performance liquid chromatographic – flow fluorometric assay for intracellular 6-thioguanine nucleotide metabolites of 6-mercaptopurine and 6-thioguanine. J Chromatogr 145: 237

Received October 6, 1980/Accepted March 11, 1981