

Pharmacology of 6-Thioguanine in Man¹

G. A. LePage² and J. P. Whitecar, Jr.

The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025

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

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²Harry Carothers Wiess Professor of Pharmacology.

³The abbreviations used are: 6-TG, 6-thioguanine; PCA, perchloric acid; 6-TX, 6-thioxanthine; 6-TU, 6-thiouric acid; 6-MeTG, 6-methylthioguanine.

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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 R_F values were as follows. With the 5% KH₂PO₄ system, the R_F value for 6-TG was 0.25; with isobutyric acid: NH₄OH:H₂O, the

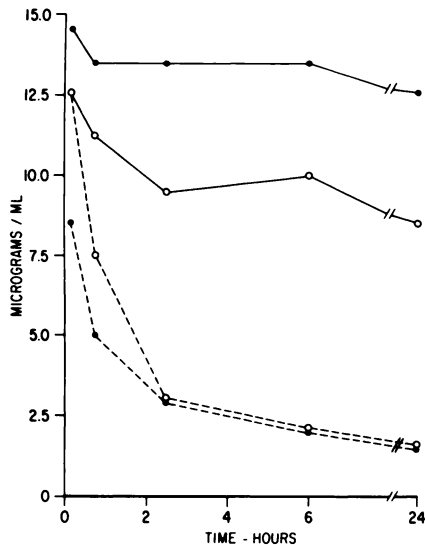


Chart 1. Levels of 6-TG and metabolites in RBC's and plasma of a patient (N. W.) with acute myelogenous leukemia given an i.v. dose of 6-TG-³⁵S at 135 mg/sq m. Total dose was 210 mg. ●—●, 6-TG + metabolites in RBC's; ○—○, 6-TG + metabolites in plasma; ●- -●, 6-TG in RBC's; ○- -○, 6-TG in plasma.

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 (R_F 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

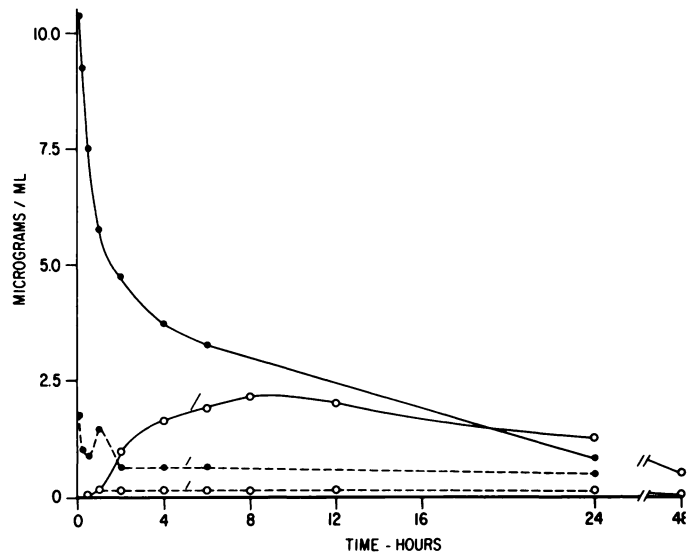


Chart 2. Levels of 6-TG and metabolites in the plasma of a solid tumor patient (J. L. K.) given 6-TG-³⁵S i.v. at 135 mg/sq m, 256 mg total dose, and 22 days later given the same dosage level p.o. ●—●, 6-TG + metabolites after an i.v. dose; ○—○, 6-TG + metabolites after a p.o. dose; ●- -●, 6-TG after an i.v. dose; ○- -○, 6-TG after a p.o. dose.

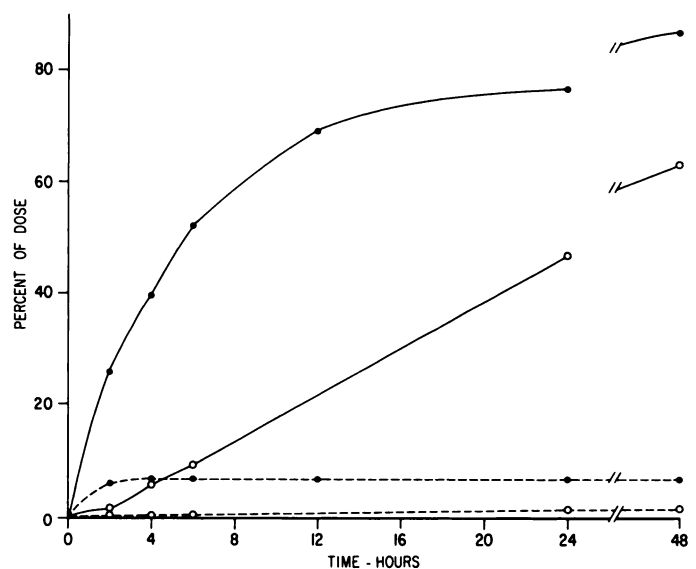


Chart 3. Urinary excretion of 6-TG and metabolites by a solid tumor patient (J. L. K., Chart 2) given 6-TG-³⁵S i.v. at 135 mg/sq m, 256 mg total dose, and 22 days later given the same dosage level p.o. ●—●, 6-TG + metabolites after an i.v. dose; ○—○, 6-TG + metabolites after a p.o. dose; ●- -●, 6-TG after an i.v. dose; ○- -○, 6-TG after a p.o. dose.

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.

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

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.

Table 2
Excretion of 6-TG and its products during and after treatment once daily
for 5 days, expressed as mg of 6-TG

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

^a Five daily doses given.

^b AML, acute myelogenous leukemia.

^c ALL, acute lymphocytic leukemia.

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 2 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 μ g 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 μ g DNA. In some cases, not analyzed, the yield was less than 15 μ g 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.

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