

## Pharmacokinetics and Metabolism of $\beta$ -2'-Deoxythioguanosine and 6-Thioguanine in Man

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**Summary.** Resistance to the antileukemic agent 6-thioguanine (TG) inevitably develops in animal tumors. However, a new agent,  $\beta$ -2'-deoxythioguanosine ( $\beta$ -TGdR) can overcome TG resistance in animal tumor models and is therefore of potential clinical use. The pharmacokinetics of radiolabeled TG were compared with those of  $\beta$ -TGdR in patients with cancer after intravenous administration. [ $^{35}$ S]- $\beta$ -TGdR (5.4 mg/kg, 200 mg/m<sup>2</sup>, 200  $\mu$ Ci total) was administered to five patients; the radiolabel in the plasma declined with an initial half-life ( $t_{1/2}$ ) of 14 min and a terminal  $t_{1/2}$  of 19.3 h. Within 24 h, 65% of the radiolabel was excreted in the urine. In contrast, after administration of [ $^{35}$ S]-6-TG (3.4 mg/kg, 125 mg/m<sup>2</sup>, 200  $\mu$ Ci total) the average initial  $t_{1/2}$  was 40 min while the terminal phase  $t_{1/2}$  was 28.9 h. Urinary excretion of the radiolabel was 75% of the dose 24 h after administration. Both thiopurines were rapidly and extensively degraded and excreted as 6-thioxanthine, inorganic sulfate, S-methyl-6 thioxanthine, and 6-thiouric acid in addition to other products. Small amounts of unchanged drug were also excreted. These studies suggest that  $\beta$ -TGdR is merely a latent form of TG.

### Introduction

The antileukemic agent 6-thioguanine (TG, NSC-752) has a tendency to induce cellular resistance in experimental tumors. TG is converted to the nucleotide 6-thioguanosine 5'-monophosphate (6-TGRP), which acts as a pseudo-feedback inhibitor of hypoxanthine-guanine phosphoribosyl transferase (EC 2.4.2.8) [7], an enzyme that is deleted in

the resistant cell line [9]. Because resistance to 6-TG may also occur in man, the deoxyribosyl derivative of TG,  $\beta$ -2'-deoxythioguanosine [9- $\beta$ -D-(2'-deoxy-ribofuranosyl)-9-H-2-aminopurine-6-thiol,  $\beta$ -TGdR, NSC-71261] was synthesized [4]. In experimental systems, this agent was capable of bypassing resistance to 6-TG by several mechanisms [8]. Concurrent with its clinical trial, we have compared the pharmacokinetics and metabolism of  $\beta$ -TGdR with those of TG in man to determine whether  $\beta$ -TGdR offers any potential therapeutic advantage over TG.

### Materials and Methods

$\beta$ -TGdR and TG, formulated as the sodium salts for intravenous (IV) administration, were supplied by the Drug Development Branch of Drug Research and Development, Division of Cancer Treatment, National Cancer Institute. Radioactive [ $^{35}$ S]- $\beta$ -TGdR (70–474  $\mu$ Ci/mmol) and [ $^{35}$ S]-TG- (52  $\mu$ Ci/mmol) were prepared by sulfur exchange of unlabeled compounds with elemental  $^{35}$ S [8]. [ $^{14}$ C]-TG (84  $\mu$ Ci/mmol) was likewise synthesized by thiation of [ $^{14}$ C]-guanine with nonradioactive sulfur. These compounds were recrystallized until their chemical and radiochemical purities exceeded 99% according to chromatographic and radiochemical analyses. 2-Hydroxy-6-methylthiopurine (S-methyl-6-thioxanthine, Me-TX) was prepared by methylation of 6-thioxanthine with methyl iodide [2, 5]. 2-Amino-6-methylthiopurine (S-methyl-6-thioguanine, Me-TG) was purchased from Aldrich Chemical Company, Milwaukee, WI, USA, and 6-thioxanthine (TX) and 6-thiouric acid (TUA) from Sigma Co., St Louis, MO, USA and Calbiochem, La Jolla, CA, USA. Allopurinol was a product of Burroughs Wellcome & Co., Research Triangle Park, NC, USA. Other chemicals and reagents were purchased from regular commercial sources.

**Radiochemical Techniques.** Radioactivity was determined with a Packard model 3385 Tricarb liquid scintillation spectrometer. Quenching, if any, was corrected by comparison of channel ratios of external standards. Plasma and urine (0.2 ml) were counted in 11 ml 'PCS,' a commercial phase-combining counting solution available from Amersham Corp., Arlington Heights, IL. Paper and thin layer chromatograms were scanned for radioactivity as described previously [2].

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\* Deceased, to whose memory this paper is dedicated

**Paper Chromatography.** Descending paper chromatography (PC) was accomplished with Whatman paper no. 3; Whatman no. 1 paper was used with solvent system B (Table 1). The solvent systems and the average  $R_f$  values of  $\beta$ -TGdR and suspected metabolites including TG, Me-TG, TX, Me-TX, TUA, and sulfate are listed in Table 1. A metabolite and an authentic compound were considered identical if they had the same  $R_f$  values in at least three solvent systems. For separation of compounds with similar  $R_f$  values, the solvent front was allowed to run over the edge of the paper. After development, the paper chromatograms were cut into  $2 \times 2$  cm squares or  $2 \times 3$  cm strips, and the radioactivity on the paper was determined directly by liquid scintillation counting.

**Ion Exchange Chromatography.** Isolation of drug metabolites from the urine of patients injected with  $[8-^{14}\text{C}]\text{TG}$  was carried out by ion exchange chromatography with Dowex-50 resin and linear gradient elution with HCl (0.5–5 N) as previously described [3]. The eluent

was monitored simultaneously for radioactivity and ultraviolet absorbance at 254 nm.

**Patients.** Patients with neoplastic diseases who were scheduled for  $\beta$ -TGdR or TG trial therapy took part in these studies; however, those patients with clinical and laboratory evidence of kidney and liver malfunction were excluded. Other cancer chemotherapy was suspended during the study. Informed written consent was obtained according to United States government guidelines. The radioactive drug, 200  $\mu\text{Ci}$  per patient, was administered IV over 10 min; the dosage of TG was 3.4 mg/kg (125 mg/m<sup>2</sup>) and that of  $\beta$ -TGdR was 5.4 mg/kg (200 mg/m<sup>2</sup>). Blood was sampled and processed as previously described [6]. Urine was collected as voided.

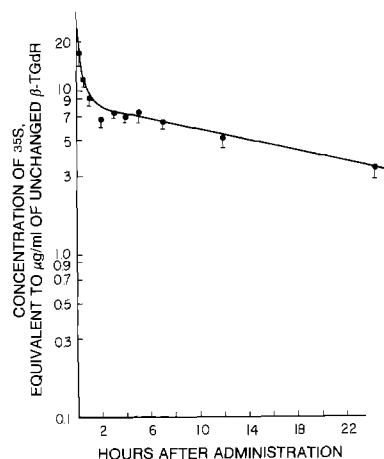
**Curve Fitting.** Curve fitting of plasma drug concentration-versus-time results was accomplished by nonlinear regression analysis, with correlation coefficients better than 0.95.

**Table 1.**  $R_f$  values of  $\beta$ -TGdR, 6TG and derivatives<sup>a</sup>

Compound	Solvent system <sup>b</sup>				
	A	B	C	D	E
$\beta$ -TGdR	0.53	0.7	0.39	0.42	0.54
TG	0.37	0.43	0.28	0.35	0.35
Me-TG	0.63	0.32	0.60	0.67	0.63
TX	0.32	0.64	0.18	0.42	0.43
Me-TX	0.74	0.18	0.79	0.54	0.52
TUA	0.25	0.58	0.09	0.31	0.30
Sulfate	0.35	1.0	0.0	0.17	0.0

<sup>a</sup> Descending paper chromatography with Whatman no. 3 (except Whatman no. 1 paper in system B) monitored by absorbance at 245 nm

<sup>b</sup> All proportions v/v. A, 95% ethanol-saturated sodium borate-5 M ammonium acetate-0.5 M sodium acetate (66 : 24 : 6 : 0-15); B, water adjusted to pH 10 with 1 N  $\text{NH}_4\text{OH}$ ; C, 95% ethanol-1 M ammonium acetate (100 : 6); D, 0.64% boric acid in 85% ethanol-conc.  $\text{NH}_4\text{OH}$  (100 : 1); E, 95% ethanol-*t*-butanol-88% formic acid- $\text{H}_2\text{O}$  (60 : 20 : 5 : 15)



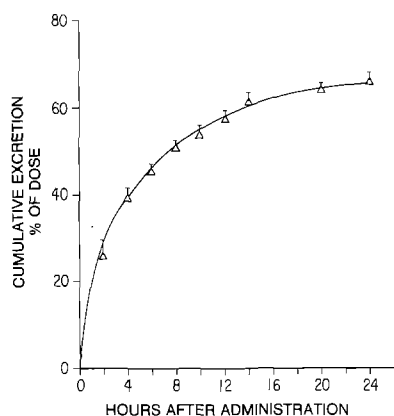
**Fig. 1.** Plasma disappearance of  $[^{35}\text{S}]$  after IV administration of  $[^{35}\text{S}]\text{-TGdR}$  to five cancer patients. Values shown are the means  $\pm$  standard errors

## Results

After IV administration of 5.4 mg/kg (200 mg/m<sup>2</sup>), the plasma clearance of  $\beta$ -TGdR and metabolites was biphasic (Fig. 1). The pharmacokinetic parameters are summarized in Table 2. Approximately 65% of the total radioactivity was excreted in the urine in 24 h (Fig. 2). The normalized percentage distribution of metabolites in the urine of a patient 1 h after  $\beta$ -TGdR-<sup>35</sup>S administration was, in decreasing order: TG, 35%;  $\beta$ -TGdR, 27%; TX, 22%; TUA, 11%; sulfate, 3%; Me-TX, 2%; and Me-TG, 1%. By the 13th h, the distribution was: TUA, 28%; Me-TG, 21%; sulfate, 20%; TX, 15%; TG, 6%;  $\beta$ -TGdR and Me-TX, 5% each (Fig. 3). In five patients, the simultaneous administration of allopurinol IV at 270 mg/m<sup>2</sup> with  $\beta$ -TGdR exerted no influence on the plasma clearance of  $\beta$ -TGdR and metabolites or the cumulative excretion of (<sup>35</sup>S) in the urine (Fig. 3). The most striking change, however, was in the normalized percentage distribution of Me-TX and Me-TG in the urine of a patient (Fig. 4). When allopurinol was administered, the relative amount of Me-TX increased while that of Me-TG decreased. The relative amounts of other metabolites remained unaltered.

Radioactive TG, 3.4 mg/kg (125 mg/m<sup>2</sup>), was administered IV to six patients; three received the <sup>14</sup>C- and three the <sup>35</sup>S-labeled drug. The disappearance curves of the isotopes from the plasma are depicted in Fig. 5. Unfortunately, no plasma specimens were available after 8 h because all participants were outpatients. Although the initial half-lives of the isotopes were somewhat different, the terminal  $t_{1/2}$  of (<sup>35</sup>S) was more than nine times as long as that of (<sup>14</sup>C). This was reflected in the total clearance: (<sup>14</sup>C) was cleared from the plasma more than six times as fast as (<sup>35</sup>S). Cumulative excretion of the radiolabel

was between 70% and 75% of the administered dose (Fig. 6). Figure 7 shows the normalized percentages of radioactive drug metabolites in the urines of a patient who had received [ $^{35}$ S]-TG IV. The distribution in the first hour urine was: TG, 70%; TX, 13%; TUA, 11%; sulfate, 4%; Me-TG and Me-TX, 1% each. By the 13th h, these percentages had changed to: Me-TG, 34%; TUA, 24%; sulfate, 18%; Me-TX, 10%; TX, 5%; and TG, 2%.



**Fig. 2.** Urinary excretion of [ $^{35}$ S] after administration of [ $^{35}$ S]- $\beta$ -TGdR. Values shown are the means  $\pm$  standard errors

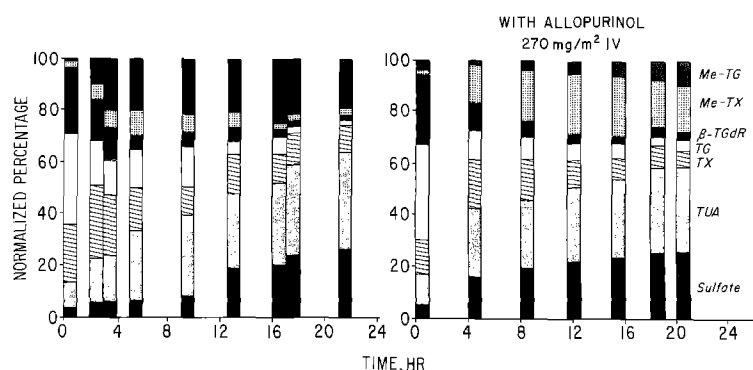
## Discussion

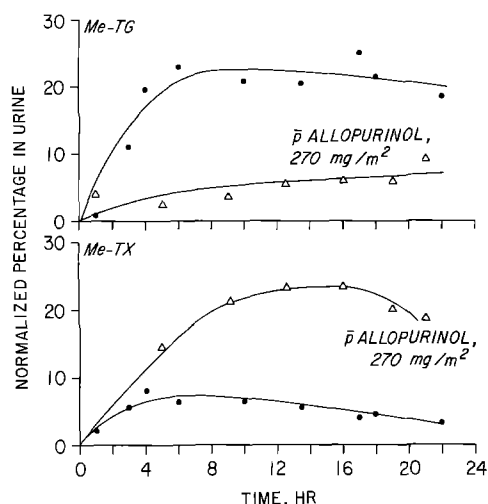
In man the terminal  $t_{1/2}$  of TG and [ $^{35}$ S]-labeled metabolites was longer than that of  $\beta$ -TGdR and metabolites; paradoxically, the plasma clearance of TG and [ $^{35}$ S]-labeled metabolites was twice that of  $\beta$ -TGdR and metabolites. This is because after a single IV administration of  $\beta$ -TGdR the area under the [ $^{35}$ S]- $\beta$ -TGdR curve ( $C \times t$ ) (Fig. 1) was more than threefold that under the [ $^{35}$ S]TG curve (Fig. 5) at equimolar dosages. Moreover, the plasma radioactivity derived from [ $^{35}$ S] $\beta$ -TGdR was higher than that from an equimolar dose of [ $^{35}$ S]TG, which is consistent with the lower cumulative excretion of  $\beta$ -TGdR and metabolites (Figs. 1 and 5; Table 2). However,  $\beta$ -TGdR may not be an effective prodrug of TG. This is apparent from the rapidly diminishing excretion of  $\beta$ -TGdR (Fig. 3), an indication of the amount of biochemically and pharmacologically available drug remaining in the body. From a clinical pharmacokinetic point of view, there are more similarities than differences between these two thio-purines. For instance, in most urine specimens, the normalized percentages of TG were comparable whether derived directly from injected TG or through the cleavage of  $\beta$ -TGdR (Figs. 3 and 7). Also, the apparent volumes of distribution of both drugs and their metabolites were in the range of 120–300 ml/kg

**Table 2.** Average ( $\pm$  standard error) plasma disappearance and cumulative urinary excretion of total radioactivity in patients after IV administration of [ $^{35}$ S]- $\beta$ -TGdR, [ $^{35}$ S]-TG, or [ $8\text{-}^{14}\text{C}$ ]-TG

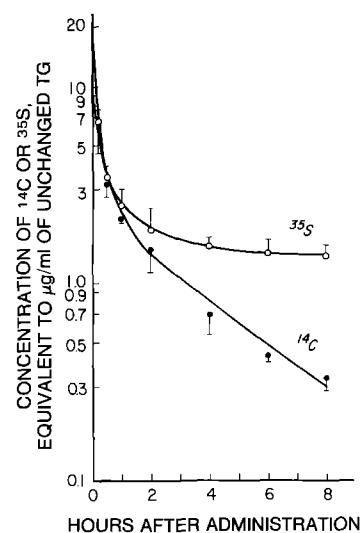
Drug	Dose (mg/m <sup>2</sup> )	No. of patients	$t_{1/2}$		VD (ml/kg)	Total clearance (ml/kg/min)	5-h urinary excretion (% of dose)
			Initial (min)	Terminal (h)			
[ $^{35}$ S]- $\beta$ -TGdR	200	5	14	19.3 $\pm$ 4.3	210 $\pm$ 6.6	0.37 $\pm$ 0.01	45 $\pm$ 5.4
[ $^{35}$ S]-TG	125	3	40	28.9 $\pm$ 0.8	148 $\pm$ 0.7	0.74 $\pm$ 0.07	54 $\pm$ 5.3
[ $8\text{-}^{14}\text{C}$ ]-TG	125	3	55	3.1 $\pm$ 0.1	262 $\pm$ 0.1	4.77 $\pm$ 0.41	43 $\pm$ 6.4

**Fig. 3.** Effect of allopurinol administration on the urinary excretion of [ $^{35}$ S]- $\beta$ -TGdR and metabolites. Values shown are the means  $\pm$  standard errors



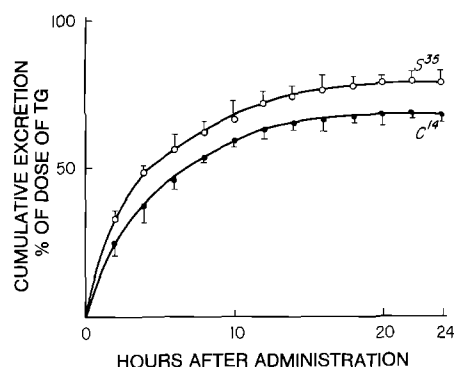


**Fig. 4.** Effect of allopurinol administration ( $\Delta$ - $\Delta$ ) on the urinary excretion of 5-methyl-6-thioxanthine (Me-TX) and 5-methyl-6-thioguanine (Me-TG). Values shown are the means  $\pm$  standard errors

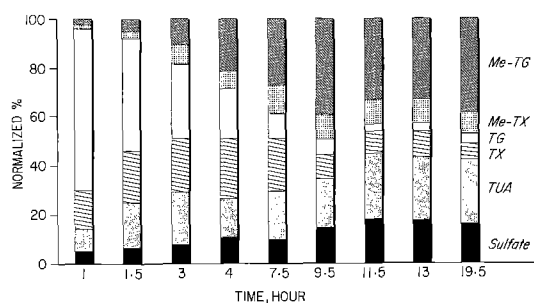


**Fig. 5.** Plasma disappearance of total radioactivity in 6 patients after administration of either ( $^{14}\text{C}$ ) or ( $^{35}\text{S}$ ) after labeled 6-TG. Values shown are the means  $\pm$  standard errors

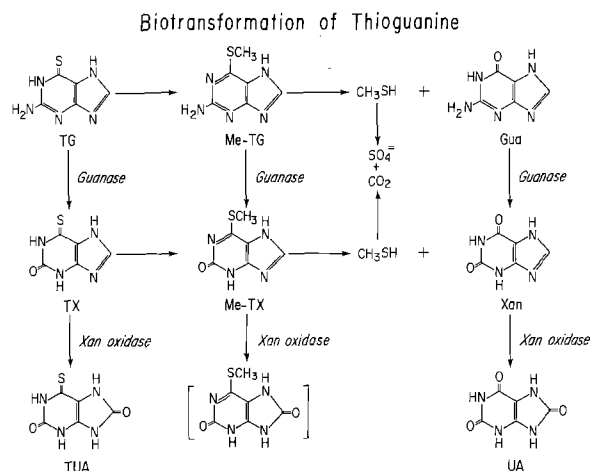
(Table 2), the sulfate space in man. Finally, both agents were rapidly and almost completely degraded in vivo, with an increasing formation of sulfate (Figs. 3 and 7). Previous studies on TG and TGdR in dogs [6] showed that the metabolism of the two thiopurines apparently follows identical pathways (Fig. 8), except that the uric acid derived from  $\beta$ -TGdR gives rise to more Me-TG in the urine. Man may be able to metabolize  $\beta$ -TGdR to Me-TG more efficiently. The most striking species differences between man and dog were in the effects of the



**Fig. 6.** Urinary excretion of ( $^{35}\text{S}$ ) or ( $^{14}\text{C}$ ) after labeled 6-TG administration. Values shown are the means  $\pm$  standard errors



**Fig. 7.** Urinary excretion of 6-TG and radiolabeled metabolites



**Fig. 8.** Metabolism of thioguanine

co-administration of allopurinol on the distribution of urinary  $\beta$ -TGdR metabolites. Though a potent xanthine oxidase inhibitor, allopurinol exerted no effect on either the *S*-methylation of TG or the deamination of TG and Me-TG. Since the rate of oxidation of TX by xanthine oxidase is at least twice that of Me-TX [3], allopurinol inhibited the oxidation of Me-TX to a

greater extent. As a result, the combination of allopurinol and  $\beta$ -TGdR caused an increased excretion of Me-TX in man (Fig. 3). In the dog, not only was the formation of Me-TG from  $\beta$ -TGdR slower than that of TX [6], but the in vivo oxidation of TX to TUA was more sensitive to allopurinol inhibition than the oxidation of Me-TX. Consequently, in dogs the simultaneous administration of allopurinol with  $\beta$ -TGdR resulted in an enhanced excretion of TX rather than Me-TX.

Although, theoretically,  $\beta$ -TGdR is capable of bypassing the several biochemical mechanisms of resistance to TG [9], there is no direct evidence that these mechanisms are operable clinically. In any event, the rapid in vivo degradation of  $\beta$ -TGdR to TG as shown by our studies clearly nullifies these considerations.

Our limited clinical trial of  $\beta$ -TGdR shows that it is active against leukemia; the undesirable side effects are moderate to severe myelosuppression and solar skin sensitization. In the absence of indisputable clinical or pharmacological evidence that  $\beta$ -TGdR is therapeutically superior to TG, the clinical trial of  $\beta$ -TGdR has now been suspended.

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