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Disposition and Metabolism of Thiopurines

III. β-2′-Deoxythioguanosine and 6-Thioguanine in the Dog

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Summary. *The anticancer agent β-2′deoxythioguanosine (β-TGdR, NSC-71261) has potential utility for the treatment of hematologic tumors resistant to 6-thioguanine (TG). We have studied the pharmacology and metabolism of these two agents in the beagle dog. [^{35}S]β-TGdR was administered as an IV bolus to five dogs at a dose of 10 mg/kg. Plasma radioactivity declined biphasically with an average terminal $t_{1/2}$ of 3.7 h. Cumulative urinary excretion of the radiolabel 5 h after administration was 19% of the total dose. In another four dogs that received 100 mg/kg (2.71 g), the average terminal plasma $t_{1/2}$ was 7.7 h and the 5-h cumulative urinary excretion was 28% of the total dose. [^{35}S]Thioguanine, 5 mg/kg was similarly administered IV to three beagle dogs. The average terminal $t_{1/2}$ of [^{35}S]TG and metabolites was 4.6 h, and the 5-h cumulative urinary excretion of the [^{35}S] label was 47%. Similar studies were conducted in three beagle dogs that received the same dose of [8^{14}C]TG. In these studies, however, the terminal phase $t_{1/2}$ of ^{14}C in plasma was 1.9 h. Cumulative urinary excretion of the ^{14}C was 40% in 5 h. Both TG and β-TGdR were rapidly and extensively degraded. Neither of these agents and none of their metabolites was found in the cerebrospinal fluid in significant concentrations. In the dog, β-TGdR was rapidly metabolized to TG and may serve as a slow release form of TG.*

Introduction

The antitumor agent 6-thioguanine (6-TG, TG, NSC-752) is used primarily for the treatment of acute leukemia [10]. It is converted by the enzyme hypoxanthine-guanine phosphoribosyltransferase to the nucleotide 6-thioguanosine 5′-monophosphate (6-TGRP), which acts as a 'pseudo'-feedback inhibitor of phosphoribosylpyrophosphate amidotransferase [8]. As is the case with antineoplastic agents which must undergo biological transformation to an activated species, cellular resistance to 6-TG often develops through a variety of mechanisms [3, 11]. In an attempt to circumvent resistance to 6-TG, a deoxyribose derivative of 6-TG,β-2′-deoxythioguanosine [9-β-D-2′-(deoxyribofuranosyl)-9-H-2-aminopurine 6-thiol, β-TGdR, NSC-71261] (Fig. 1) was synthesized [5]. β-TGdR is active against some transplantable tumors resistant to 6-TG [1]. We have compared the pharmacologic disposition and metabolism of β-TGdR with 6-TG in the beagle dog to evaluate whether β-TGdR has any potential therapeutic advantage over 6-TG from a pharmacologic point of view.

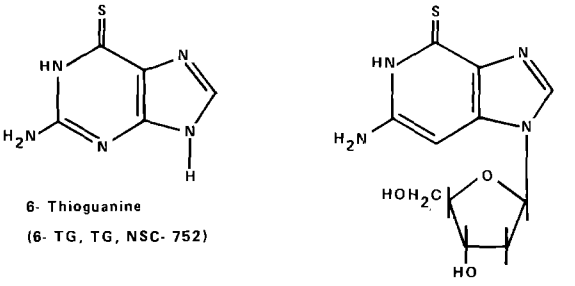

Fig. 1. Structure of 6-thioguanine and β-2[1] deoxythioguanosine

Materials and Methods

β-TGdR and TG formulated as the sodium salts for intravenous (IV) administration were supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute. Radioactive [^{35}S] β-TGdR (70 μCi/mmole) and [^{35}S]TG (52 μCi/mmole) were prepared by sulfur exchange of unlabeled compounds with elemental ^{35}S [9]. Similarly, [8-^{14}C]TG (84 μCi/

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mmole) was synthesized by thiation of guanine-8-^{14}C with nonradioactive sulfur. These compounds were recrystallized until their chemical and radiochemical purities exceeded 99% as estimated by 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, 6]. 2-Amino-6-methylthiopurine (S-methyl-6-thioguanine, Me-TG) was purchased from Aldrich Chemical Company, Milwaukee, WI, and 6-thioxanthine (TX) and 6-thiouric acid (TUA) from Sigma Co., St Louis, MO, USA and Calbiochem, La Jolla, CA, USA respectively. Allopurinol was a product of Burroughs Wellcome Company, 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 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/Searle Corp., Arlington Heights, IL, USA. Paper and thin layer chromatograms were scanned for radioactivity as previously described [2].

Paper Chromatography. Descending paper chromatography (PC) was accomplished with Whatman Filter Paper. The solvent systems and the average R_f values of β-TGdR and possible metabolites, including TG, Me-TG, 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 further 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 was cut into 2 cm × 2 cm squares or 2 cm × 3 cm strips, and the radioactivity was determined as before.

Ion Exchange Chromatography. Isolation of drug metabolites from the urine of dogs injected with [8-^{14}C] TG was carried out by ion exchange chromatography with Dowex-50 resin and linear gradient elution with HCl (0.5 N–5 N), as previously described [4]. The eluent was monitored simultaneously for radioactivity and ultraviolet absorbance at 254 nm.

Table 1. R_f values of β-TGdR and derivatives[a]

Compound	Solvent system[b]				
	A	B	C	D	E
β-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

[a] Descending paper chromatography with Whatman no. 3 paper (except Whatman no. 1 paper in system B)

[b] 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 NH_4OH; C, 95% ethanol–1 *M* ammonium acetate (100 : 6); D, 0.64% boric acid in 85% ethanol–conc. NH_4OH (100 : 1); E, 95% ethanol–*t*-butanol–88% formic acid–H_2O (60 : 20 : 5 : 15)

Protein Binding. Binding of β-TGdR and 6-TG to plasma protein was determined by ultrafiltration with an Amicon apparatus [7]. Each experiment was repeated at least three times. Human plasma was obtained from the blood bank of our institution; dog plasma was prepared from the pooled blood of canine donors. Bovine serum albumin (BSA) power, fraction V, was purchased from Schwarz/Mann, Orangeburg, NY, USA.

Ultraviolet Absorption Spectrometry. Ultraviolet absorption spectra were recorded with a Cary model 14 recording spectrophotometer.

Dogs. Beagle or mongrel dogs of either sex, weighing 10–18 kg, were lightly anesthetized with pentobarbital. Radioactive β-TGdR or TG, both as the sodium salts, was administered through the femoral vein in 10 min. Each dog received 100–200 μCi of radioactivity. The 6-TG dose was 5 mg/kg to five dogs, and 100 mg/kg to four dogs. Blood was sampled at intervals, and heparin was used as an anticoagulant. The plasma was separated from the cells by centrifugation at 12,000 *g* for 10 min in a Sorvall RC-2β centrifuge. An indwelling Foley catheter was used for urine collection. The bladder was flushed with normal saline each time, and the flushings were combined with the appropriate urine sample. The cerebrospinal fluid (CSF) was sampled by cisternal puncture.

Results

The disappearance of β-TGdR and metabolites from the plasma of dogs given this agent IV at a dose of 10 mg/kg was biphasic, with an average initial $t_{1/2}$ of 7 min and a terminal $t_{1/2}$ of 3.7 h (Fig. 2). Other pharmacokinetic parameters are listed in Table 2. The pharmacokinetic parameters of unchanged β-TGdR and metabolites were not determined because plasma β-TGdR metabolites were not separable due to their low specific activity. At a [^{35}S]β-TGdR dose of 100 mg/kg, the plasma $t_{1/2}$ of β-TGdR and metabolites was considerably longer; however, the total clearance showed no significant change. The average cumulative urinary excretion of total radioactivity was about 19% of the administered dose in dogs that received 10 mg β-TGdR/kg (Table 2), but 28% in animals that received the higher dose of this agent (Fig. 3). The urine samples were analyzed for unchanged drug and metabolites; in one study the normalized percent distribution of β-TGdR and its biotransformation products in the first-hour urine were: TG, 50%; TUA, 18%; β-TGdR, 16%; TX, 6%; Me-TX, 5%; sulfate, 3%; and Me-TG, 2%. These percentages were very different from those of the 5th-h urine: TUA, 45%; TG, 17%; TX, 9%; sulfate, 16%; the remaining radioactivity was distributed equally among β-TGdR, Me-TG, and Me-TX (Fig. 4). In 5 h, therefore the relative amount of TUA excreted in the urine more than doubled, with a corresponding three-fold reduction in the amount of

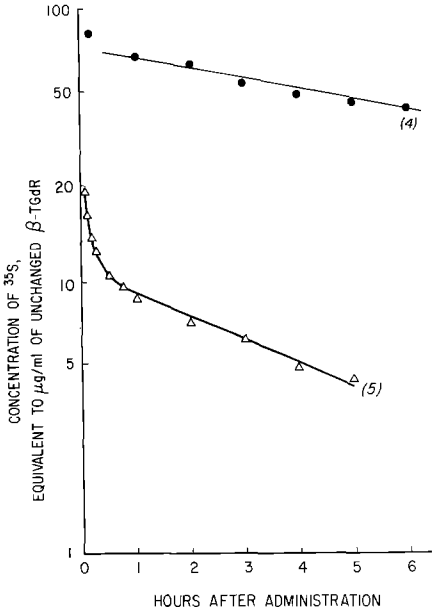

Fig. 2. Plasma clearance of [^{35}S] after IV administration of [^{35}S]β-TGdR in anesthetized dogs at a dose of 10 mg/kg (△——△, five dogs) or 100 mg/kg (●——●, four dogs)

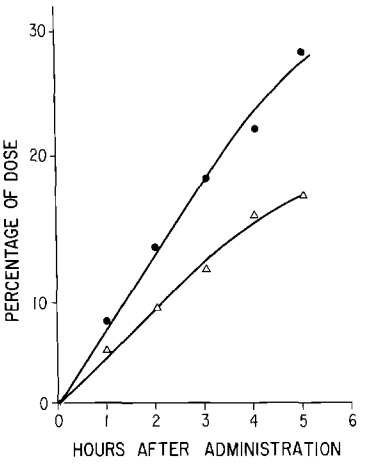

Fig. 3. Average cumulative urinary excretion of total radioactivity after IV administration of [^{35}S]β-TGdR at doses of 10 mg/kg (△——△) and 100 mg/kg (●——●)

Table 2. Pharmacokinetics of thiopurines in beagle dogs

Thiopurine	(mg/kg)	(mg/m^2)	No. of studies	$t_{1/2}$		Vd (ml/kg)	Clearance (ml · kg^{-1} · min^{-1})	Urinary excretion (% of dose)
				Initial (min)	Terminal (h)			
β-TGdR-^{35}S	10	271	5	7	3.7 ± 0.2	639 ± 155	2.8 ±	19 ± 6.0
	100	2,620	4	–	7.7 ± 1.9	1,370 ± 62	2.1 ±	28 ± 7.0
TG-^{35}S	5	124	3	4	4.6 ± 0.5	370 ± 27	0.7 ±	47 ± 0.6
TG-8-^{14}C	5	138	3	6	1.9 ± 0.1	520 ± 41	4.8 ±	40 ± 2.3

TG. Also evident were similar changes in the excretion of β-TGdR and inorganic sulfate.

Because purine nucleoside phosphorylase (EC 2.4.2.1) and xanthine oxidase (EC 1.2.3.2) are involved in the stepwise degradation of β-TGdR ultimately to TUA, we have investigated the possible effects of the simultaneous administration of hypoxanthine or allopurinol on the pharmacokinetics of β-TGdR. In one of the dogs that received 50 mg [^{35}S]β-TGdR/kg, the same dose of β-TGdR-^{35}S] was administered 1 week later together with 63 mg hypoxanthine/kg, a substrate of purine nucleoside phosphorylase [4]. At this dosage, hypoxanthine had no effect on the disposition and metabolism of β-TGdR. Similar experiments were carried out in another dog but with instead 56 mg allopurinol/kg (a potent xanthine oxidase inhibitor). There was no appreciable change in the plasma clearance of β-TGdR and metabolites; however, the 5-h cumulative excretion of radioactivity showed a decrease from 41% to 33%. Moreover, the relative amount of TX was noticeably elevated and that of TG slightly higher in all urine samples. These changes were accompanied by a corresponding reduction in excretion of TUA and a lesser reduction of sulfate (Figs. 4 and 5). The excretion patterns of other metabolites remained about the same (Fig. 4).

The pharmacokinetics of TG were found to differ from those of β-TGdR in several aspects (Table 2). In two groups of three dogs each, after 5 mg [^{35}S]TG or [8-^{14}C]TG/kg IV the plasma clearance of ^{35}S or ^{14}C was also biphasic (Fig. 6 and Table 2). The cumulative urinary excretion of total isotopes is shown in Fig. 7. The normalized percent distribution of TG

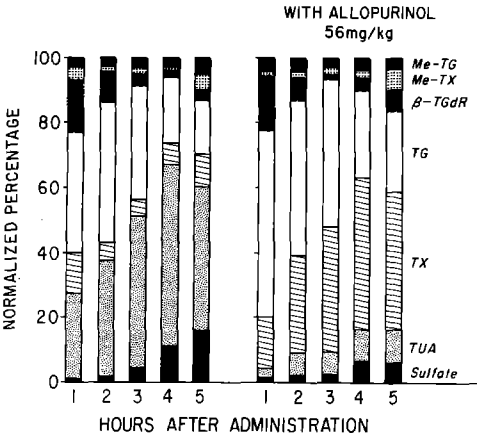

Fig. 4. Distribution of [^{35}S] in the urine of a dog after IV injection of [^{35}S]β-TGdR (50 mg/kg)

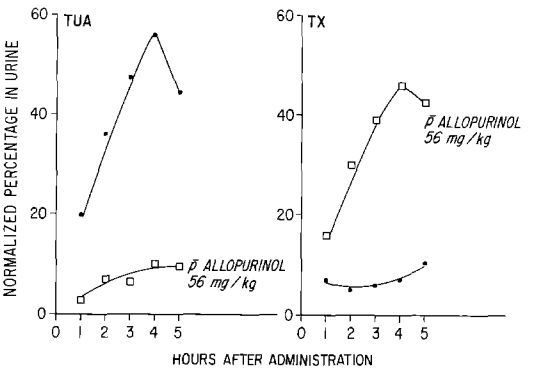

Fig. 5. The effect of allopurinol on the metabolism of [^{35}S]β-TGdR after IV administration (50 mg/kg)

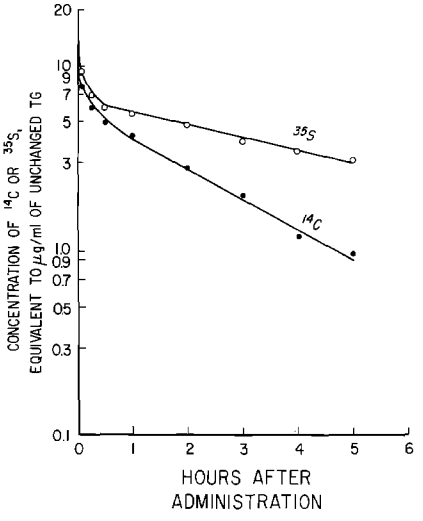

Fig. 6. Plasma clearance of radioactivity after IV administration of either [^{14}C]- (one dog) or [^{35}S]-labeled (two dogs) 6-TG (5 mg/kg)

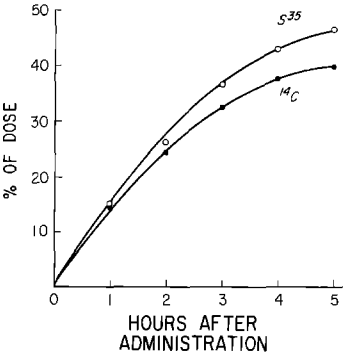

Fig. 7. Cumulative urinary excretion of radioactivity after administration of either [^{14}C]-(one dog) or [^{35}S]-labeled (two dogs) 6-TG (5 mg/kg)

and metabolites in the urine samples of a dog at 1 h after IV administration of 5 mg [^{35}S]TG/kg is depicted in Fig. 8: TG, 62%; TUA, 23%; sulfate, 10%; TX, 5%; and Me-TG, 1%; at 5 h, TUA, 38%; Me-TG, 32%; sulfate, 15%; TX, 11%; Me-TX, 4%; and TG, 1%. The 3-h urine of another dog that had received the same dose of [8-^{14}C]TG was analyzed by ion-exchange chromatography, and the elution profile is illustrated in Fig. 9. In this chromatographic system uric acid would appear in the first prominent peak. Since the injected [8-^{14}C]TG gave rise to radioactive uric acid (UA), xanthine (XAN), and guanine (GUA) through the loss of sulfur, their amounts in

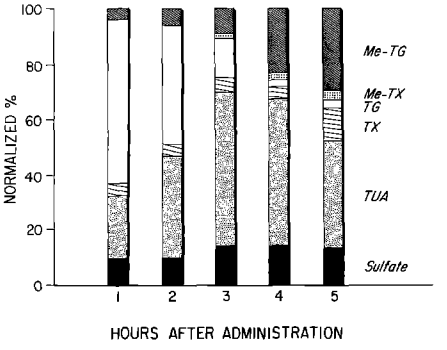

Fig. 8. Distribution of the [^{35}S] label in urine after IV injection of [^{35}S]-labeled 6-TG (5 mg/kg)

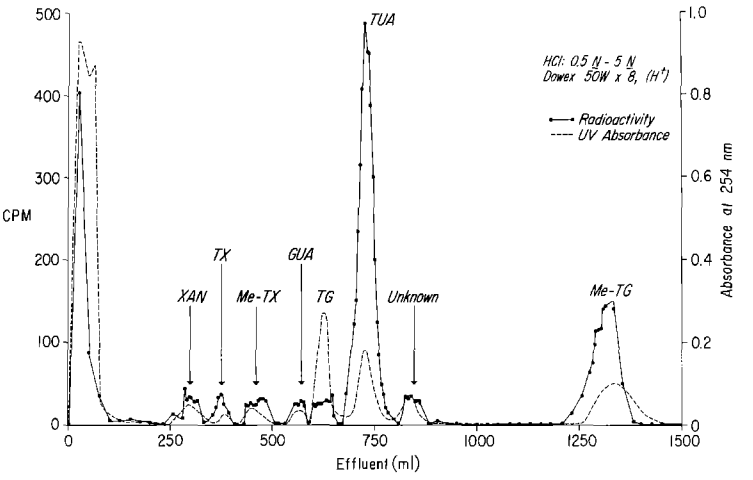

Fig. 9. Cation exchange chromatogram of dog urine 3 h after administration (IV) of [^{14}C]-labeled 6-TG (5 mg/kg, 26 μCi total dose)

this urine sample may be taken together as equivalents of sulfate. Thus, the normalized distribution of various metabolites were approximately: TUA, 40%; sulfate, 27%; Me-TG, 22%; Me-TX, 3%; TG, 3%; TX, 2%; and an unknown, 3%. Compared with the results of the previous dog (Fig. 8), there was more Me-TG, and possibly more sulfate, in this urine specimen.

At concentrations of 0.5–60 μg/ml (1.8–212 μ*M*), β-TGdR was 6%–42% bound to plasma protein; the binding constants at 25° were: human plasma, $7.1 \pm 4.4 \times 10^3/M$; and BSA, $6.6 \pm 3.4 \times 10^3/M$. In contrast, 6-TG was less bound in the same drug concentration range, being 1%–15% bound with the following binding constants: human plasma, $7.4 \pm 4.9 \times 10^2/M$; dog plasma, $3.0 \pm 1.8 \times 10^3/M$; BSA, $4.8 \pm 1.6 \times 10^3/M$. In these experiments, we assume: first, protein albumin was the only fraction involved in the binding; second, all the albumins had a molecular weight of 6.5×10^4 daltons; third, the BSA was homogenous.

No significant radioactivity was detected in the CSF of dogs that received injections of either [^{35}S]TG or [^{35}S]β-TGdR.

Discussion

Both 6-TG and β-TGdR apparently undergo cleavage at the C-S bond in vivo, forming metabolites which may readily be incorporated into endogenous purine and sulfate pools (Figs. 4 and 8). In the case of [^{35}S]TG, the purine base was not radioactive and therefore [^{35}S]sulfate was the only cleavage product detected. The terminal plasma $t_{1/2}$ of ^{35}S in the dog after [^{35}S]TG or β-TGdR administration was 4.6 h, similar to that observed after $Na_2\ ^{35}SO_4$ administration (5.3 h). It is possible that plasma [^{35}S] activity after 6-TG or β-TGdR administration during the terminal phase may be largely contributed to by sulfate. On the other hand, compared with [^{35}S]TG, the facile utilization of labeled purines derived from the metabolism of [8-^{14}C]TG readily accounts for the shorter plasma $t_{1/2}$ (1.9 h) and the higher urinary excretion of radioactivity (Table 2). Moreover, the relatively high total clearance of ^{14}C-labeled metabolities is similar to the clearance of urate in the dog [9].

The simultaneous administration of allopurinol with β-TGdR resulted in enhanced excretion of TX rather than Me-TX. This may suggest that the formation of Me-TX from β-TGdR is slower than the formation of TX or that the in vivo oxidation of TX and TUA is more sensitive to allopurinol inhibition than the oxidation of Me-TX. In common with other purine and purine nucleoside analogs, neither 6-TG nor β-TGdR was extensively bound to plasma protein at therapeutic concentrations. Furthermore, 6-TG and β-TGdR did not reach the central nervous system with ease, and may therefore have limited clinical utility for the treatment of intracranial tumors.

Although β-TGdR is theoretically capable of bypassing biochemical mechanisms of resistance to 6-TG, there is no evidence that these mechanisms are operable clinically. In any event, the rapid in vivo degradation of β-TGdR to TG, as shown in our studies, clearly nullifies these considerations. On the other hand, β-TGdR may serve as a depot form of TG, and may thereby offer some theapeutic advantages over 6-TG.

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References

1. Barranco SC, Humphrey RM (1971) The effects of β-2′-deoxythioguanosine on survival and progression in mammalian cells. Cancer Res 31: 583
2. Bergman F, Kwietny-Govrin H, Ungar-Waron H, Kalmus A, Tamari M (1963) Relation of structure to the inhibitory activity of purines against urate oxidase. Biochem J 86: 567
3. Brockman RW (1963) Mechanisms of resistance to anticancer agents. Adv Cancer Res 7: 129
4. Housholder GE, Loo TL (1971) Disposition of 5-(3,3-dimethyl-1-triazeno)-imidazole-4-carboxamide, a new antitumor agent. J Pharmacol Exp Ther 179: 386
5. Iwamoto RH, Acton EM, Goodman L (1963) 2'-Deoxythioguanosine and related nucleotides. J Med Chem 6: 684
6. Leonard EO, Orme-Johnson WH, McMurtray TR, Skinner CG, Shieve W (1962) 2-Hydroxy derivatives of some biologically active 6-(substituted) purines. Arch Biochem Biophys 99: 16
7. Loo TL, Luce JK, Sullivan MP, Frei E III (1968) Clinical pharmacological studies of 6-mercaptopurine and 6-methylthiopurine ribonucleoside. Clin Pharmacol Ther 9: 1980
8. McCollister RJ, Gilbert WR, Ashton DM, Wyngaarden JB (1964) Pseudofeedback inhibition of purine synthesis by 6-mercaptopurine ribonucleotide and other purine analogues. J Biol Chem 239: 1560
9. Moravek J, Nejedly Z (1960) Labeling of 6-mercaptopurine and 6-mercaptopurine riboside by an exchange reaction with elementary sulphur-^{35}S. Chem Ind 530
10. Pinkel D (1978) Chemotherapy of acute leukemia. In: Brodsky I, Kahn SB, Conroy JF (eds) Cancer chemotherapy, vol 3. Grune & Stratton, New York, p 429
11. Wolpert MK, Damle SP, Brown JE, Sznycer E, Agrawall KC, Sartorelli AC (1971) The role of phosphohydrolase in the mechanism of resistance of neoplastic cells to 6-thiopurine. Cancer Res 31: 1620

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