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Letter to the Editor

Lack of Activity of β-2′-Deoxythioguanosine against Two Tumors Resistant to 6-Thioguanine[1]

β-TGdR[2] was introduced by LePage *et al.* (4, 5, 7) as an antitumor agent that may offer therapeutic advantages over 6-TG. On the basis that incorporation of 6-TG into DNA is the mechanism for antitumor activity (3), cells deficient in HGPRTase (hence, resistant to 6-TG) would be sensitive to β-TGdR if the deoxyribonucleoside were phosphorylated by deoxyguanosine kinase (5, 7). The 2 HGPRTase-deficient tumors tested demonstrated marginal sensitivity toward β-TGdR at best (7). Another hypothesized mechanism of resistance to 6-TG, presumably operative in the Mecca lymphosarcoma, involves inability of cells to form deoxyribonucleotides from 6-thio-GDP (7). Whether or not this mechanism of resistance exists is subject to experimental proof; however, β-TGdR is only slightly (15% increase in survival time) active against this 6-TG-resistant tumor as well (7).

We have tested β-TGdR for activity against 2 experimental tumors that are deficient in HGPRTase activity. The tumors, H. Ep. 2/MP and L1210/MP, are clearly resistant to 6-TG and to 6-MP due to the absence of this enzyme. Clear-cut cross-resistance to β-TGdR was observed. In the L1210/MP cell line, [^{35}S]-β-TGdR was not appreciably accumulated by the cells. This suggests that the major route for conversion of β-TGdR to nucleotides does not involve deoxyguanosine kinase and that β-TGdR is converted to 6-TG by the action of PNPase with subsequent ribosylphosphorylation of 6-TG by means of HGPRTase. Questions are raised concerning the practical advantages of the clinical use of β-TGdR instead of 6-TG since the therapeutic index may be similar (7) and the incidence of side effects in man may be greater (4).

Methods. Human epidermoid carcinoma cells of the line (H. Ep. 2) established in culture by Moore *et al.* (8) were tested for sensitivity to 6-TG and β-TGdR by the cloning technique previously described (14). Briefly, about 100 cells in 10 ml of SRI-14 medium (2) are allowed to form colonies during 7 to 14 days of incubation in the presence or absence of drug. After staining, macrocolonies are counted and cloning efficiency is compared for controls and drug-treated samples. L1210/0 and L1210/MP are maintained by the weekly transfer of cells harvested from the i.p. culture in female C57BL × DBA/2 (hereafter called $BD2F_1$) mice. The chemotherapy evaluation and maintenance of these cells has been reviewed (13) as has the loss of HGPRTase in the 6-MP-resistant L1210 and H. Ep. 2 tumors (1).

β-TGdR was kindly supplied by Dr. Harry B. Wood, Jr., of the National Cancer Institute. [^{35}S]-β-TGdR was prepared from this sample by radioisotopic exchange with elemental ^{35}S (9) by New England Nuclear, Boston, Mass. The radiochemical purity of the labeled compound was greater than 94% as determined by paper chromatography in 1-butanol:acetic acid:water (4:1:1).

Incorporation of [^{35}S]-β-TGdR into cells or into the RNA and DNA of cells was determined as previously described (12). Essentially, an aliquot of washed cells is used for determination of total cellular uptake by liquid scintillation counting, and another aliquot is treated with trichloroacetic acid for measurement of total acid-insoluble (RNA + DNA) radioactivity. The acid-insoluble material remaining after treatment of the acid precipitate with 0.4 N NaOH is used as a measure of DNA incorporation. We have used 5-fluorodeoxyuridine treatment in these experiments to verify that the radioactivity from [^{35}S]-β-TGdR in acid-insoluble, NaOH-stable material is reduced by inhibi-

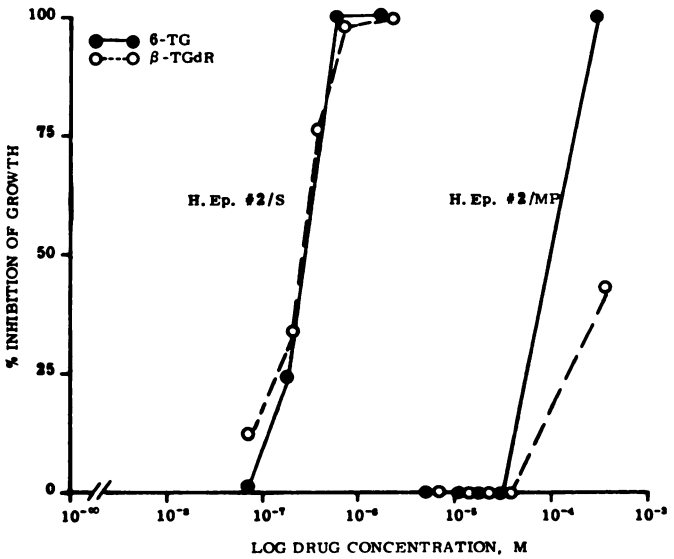

Chart 1. Cross-resistance of H. Ep. 2/MP cells to 6-TG and to β-TGdR. H. Ep. 2/S, a cell line sensitive to 6-MP, and H. Ep. 2/MP, a cell line selected for resistance to 6-MP, were allowed to form clones in the presence of 6-TG or β-TGdR at the concentrations shown. Inhibition of growth refers to the cloning efficiency of treated cells compared to that of controls.

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[2] The abbreviations used are: β-TGdR, β-2′-deoxythioguanosine; 6-TG, 6-thioguanine; HGPRTase, hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8); 6-MP, 6-mercaptopurine; PNPase, purine nucleoside orthophosphate ribosyltransferase (EC 2.4.2.1).

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Table 1

Resistance of a L1210 ascites tumor lacking HGPRTase to β-TGdR

Dose[a] (mg/kg/dose)	L1210/0[b] Mean life-span (days)	L1210/0[b] % ILS[e]	L1210/MP[c] Mean life-span (days)	L1210/MP[c] % ILS	Toxicity control, 60th-day survivors[d]
No drug control	9.7		11.6		10/10
0 6	14.1	+45	11.6	0	10/10
0.9	14.6	+50	11.8	+1	10/10
1.35	14.3	+47	11.6	0	10/10
2.0	12.9	+32	11.5	−1	10/10
3.0	14.2	+46	9.8	−16	10/10
4.5	>20.2	>+100	9.5	−19	8/10

[a] Drugs administered i.p. 1 time daily for 9 days beginning 1 day after implantation of cells.
[b] 10^5 cells implanted i.p.
[c] 10^6 cells implanted i.p.
[d] Non-tumor-bearing $BD2F_1$ female control mice.
[e] ILS, increased life-span.

Table 2

Uptake of β-TGdR by a sensitive (L1210/0) and resistant (L1210/MP) ascites tumor

Mice bearing 4-day implants of tumor were treated in groups of 3 with [^{35}S]-β-TGdR (90 μCi/μmole; 8 μCi/mouse) alone or 30 min after a dose of FUdR[a] of 250 mg/kg. Two hr after giving the [^{35}S]-β-TGdR, cells were harvested and washed, and radioactivity was determined in the cells (total) or extracts prepared as previously described (12).

Extract	[^{35}S]-β-TGdR-incorporated (nCi/10^7 cells) Alone (A) L1210/0	Alone (A) L1210/MP	+ FUdR (B) L1210/0	+ FUdR (B) L1210/MP	FUdR-sensitive, A − B L1210/0	FUdR-sensitive, A − B L1210/MP
Total	35.2	5.26	9.82	3.94	25.4	1.32
Acid-insoluble	18.8	1.13	3.42	0.47	15.4	0.66
Acid-insoluble, NaOH-stable	9.0	0.50	1.78	0.18	7.2	0.32

[a] FUdR, 5-fluorodeoxyuridine.

tion of DNA synthesis. The dose of 5-fluorodeoxyuridine used was shown to produce extensive inhibition of DNA synthesis using [*methyl*-^{3}H]thymidine as a precursor.

Results and Discussion. On a molar basis, 6-TG and β-TGdR are equally effective as inhibitors of H. Ep. 2/S cell growth (Chart 1). The H. Ep. 2/MP cell line deficient in HGPRTase activity is resistant to both 6-TG and to β-TGdR. β-TGdR is known to be a substrate for PNPase (6), and the cross-resistance implies that conversion of β-TGdR to 6-TG is the route for anabolism to fraudulent nucleotides. The PNPase activity in 100,000 × *g* supernatants of H. Ep. 2/S and H. Ep. 2/MP cells was high and about the same, *i.e.*, ~450 nanomolar units/ml cells using 0.1 mM deoxyguanosine as substrate.

L1210/MP cells that lack HGPRTase are resistant to β-TGdR (Table 1). β-TGdR is about as effective as 6-TG against the parent L1210/0 line (11). The absence of activity of β-TGdR against L1210/MP is probably due to the fact that these cells fail to take up appreciable quantities of the drug when compared to the parent cell line (Table 2).

The reason for apparent lack of deoxyguanosine kinase activity in whole cells of the tumors examined is not known. The kinase activity at a high level of β-TGdR (1 mM) was found to be about 7 nanomolar units/ml cells in 100,000 × *g* supernatants of L1210/0 or L1210/MP cells when measured by the assay similar to that of Nakai and LePage (10). It appears likely that in whole cells the conversion of β-TGdR to 6-TG occurs rapidly compared to the kinase reaction or that the affinity of the deoxyguanosine kinase for β-TGdR is too low (10) for good activity toward therapeutic drug levels.

In experiments not shown, Dr. Robert F. Pittillo has demonstrated cross-resistance of *Escherichia coli* ATCC 9637/6-TG to β-TGdR. This cell line has been selected for resistance to 6-TG, but the biochemical basis has not been established.

Careful examination of the reported effectiveness of β-TGdR against cells resistant to 6-TG reveals a very low order of activity (7). In light of the published data available, it appears questionable whether or not β-TGdR has significant activity against 6-TG-resistant neoplasms.

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