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MECHANISM OF RESISTANCE TO THE ONCOLYTIC C-NUCLEOSIDE 2-β-D-RIBOFURANOSYLTHIAZOLE-4-CARBOXAMIDE (NSC-286193)

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The C-nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide (TR; tiazofurin; riboxamide) exhibits significant antitumor activity against the L1210 and P388 leukemias, and is curative when tested against the Lewis lung carcinoma in mice (1). We have demonstrated recently that TR is anabolized *in vivo* to a dinucleotide in which the nicotinamide of NAD has been replaced by thiazole-4-carboxamide (2); this anabolite, hereafter referred to as TAD, is a potent inhibitor of IMP dehydrogenase both *in vitro* and *in vivo* (2-4).

A variant of P388 leukemia has now been rendered resistant to TR in culture by step-wise increases from 1 μM to 10 mM in the concentrations of TR to which the cells were exposed over a period of 60 generations. As shown in Fig. 1, this procedure resulted in a greater than

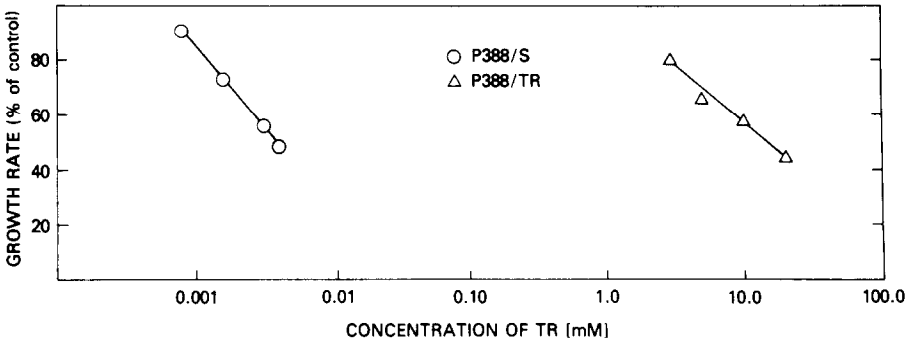

Fig. 1. Effect of TR on proliferation rate of P388/S and P388/TR cells. P388/S or P388/TR cells in log growth phase (1-5 x 10^5 cells/ml) were incubated with TR at the concentrations indicated for 24 hr at 37° in an atmosphere of 95% air and 5% CO_2. Growth medium was RPMI 1640 containing 10% calf serum, 2 mM L-glutamine and 5 μM 2-mercaptoethanol. In control flasks (TR omitted), there was a 3.5-fold increase in cell number at 24 hr under these conditions.

4-log decrease in the sensitivity of the cells to a 24-hr exposure to TR; this variant line (P388/TR) has now been propagated for over 50 generations in the absence of drug and exhibits a doubling time of 13-14 hr, a proliferation rate almost identical to that of the sensitive line (P388/S).

On transplantation of the resistant line to BDF_1 mice, stable resistance was retained with no selection pressure. As shown in Table 1, mice receiving 10^6 cells of either P388/S or P388/TR i.p. survived for ca. 10 days; TR (100 or 400 mg/kg) produced a significant increase in survival time in animals bearing the P388/S tumor but not in animals bearing the P388/TR tumor.

Table 1. Survival time of TR-treated mice bearing P388 leukemia*

Treatment	Dose (mg/kg/day)	Tumor			
		P388/S		P388/TR	
		Mean survival time (days ± S.D.)	% ILS	Mean survival time (days ± S.D.)	% ILS
Saline	-	10.8 ± 1.4	100	10.4 ± 2.2	100
TR	100	14.7 ± 0.7†	136	10.0 ± 0.7	96
	400	20.5 ± 1.9†	190	9.2 ± 0.4	89

*Groups of seven male BDF_1 mice (20-25 g) were given 10^6 P388/S or P388/TR cells i.p. At 24 hr, treatment was started by i.p. administration of TR at the doses indicated daily for 5 days.

†Significantly different ($P < 0.01$) from control values in saline-treated animals.

In view of our earlier observation (2) that the pharmacological effects of TR result from its anabolic conversion to TAD, studies were performed to compare the formation of TAD in the TR-sensitive and resistant P388 lines, both in vitro and in vivo. P388 cells in culture were exposed to [5-^{3}H]TR (10 μM) for 2 hr, and the cell extracts were subjected to chromatography on Partisil-10 SAX resin. As shown in Fig. 2, TAD was the most abundant metabolite seen in the P388/S cells, whereas it was undetected in P388/TR. In addition,

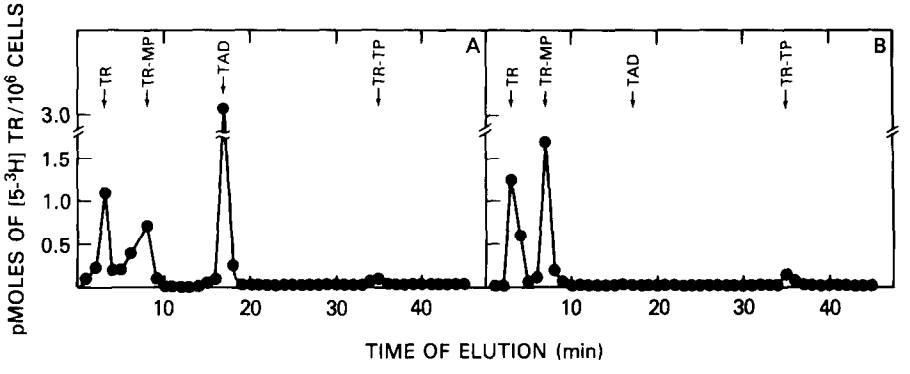

Fig. 2. Chromatographic resolution of radioactivity after exposure of P388 cells to [5-^{3}H]TR. To 50 ml of P388/S (panel A) or P388/TR (panel B) cells (ca. 5 x 10^7 cells) in RPMI 1640 medium with 3% L-glutamine, 10% calf serum and 5 μM 2-mercaptoethanol, 50 μl of [5-^{3}H]TR (500 nmoles, 34 μCi) was added. After incubation at 37° for 2 hr in an atmosphere of 95% air and 5% CO_2, cells were separated by centrifugation at 3,000 g for 2 min and washed twice with 1 ml of Hanks' balanced salt solution. Perchloric acid (PCA), 0.2 ml of a 5% solution, was then added and the cell pellet was mixed and centrifuged at 12,000 g for 2 min. The PCA extract was neutralized with 40% KOH and recentrifuged, and an aliquot was chromatographed on Partisil-10 SAX resin as previously described (3).

a 2- to 3- fold increase in the level of TR-5'-monophosphate was seen (1.1 pmoles of TR-5'-monophosphate/10^6 P388/S cells vs. 2.3 pmoles/10^6 P388/TR cells): both observations are compatible with a loss or significant decrease of the enzyme activity responsible for the conversion of the TR-5'-monophosphate to the TAD anabolite. In studies carried out in vivo, mice bearing P388/S or P388/TR tumors as subcutaneous nodules were given [5-^{3}H]TR (100 mg/kg, i.p.), the animals were killed at 1 hr, tumors were excised, and the extracts were subjected to HPLC; experimental conditions are described in Table 2. Most noteworthy is the finding that the concentration of TAD in the excised P388/S tumors was 11.5-fold higher than that measured in extracts from P388/TR tumors.

Table 2. Metabolism of TR by P388 leukemia in vivo*

Drug metabolite	Metabolite concentration in tumor (nmoles/g wet wt of tissue)		P388/S / P388/TR
	P388/S	P388/TR	
TR	22.5 ± 3.27†	11.65 ± 2.55†	1.9
TR-4-COOH	0.21 ± 0.05	0.15 ± 0.06	1.4
TR-MP	1.35 ± 0.44	0.43 ± 0.17	3.1
TAD	1.26 ± 0.38	0.11 ± 0.04	11.5
TR-DP	0.05 ± 0.02	0.03 ± 0.01	1.7
TR-TP	0.06 ± 0.01	0.04 ± 0.02	1.5

*Groups of five mice bearing P388/S or P388/TR s.c. were injected i.p. with 100 mg/kg [5-^{3}H]TR (10 μCi/mg). One hour later, tumors were removed, flash frozen on dry ice, and homogenized in 5% PCA. Supernatant fractions were neutralized with 40% KOH and aliquots were chromatographed on Partisil-10 SAX resin as previously described (3).

†Mean ± S.D.

We have demonstrated previously (2) that TAD is a potent inhibitor of IMP dehydrogenase, a key step in purine nucleotide biosynthesis (5), and also that administration of TR results in a notable depression of the concentration of all guanine nucleotides, consequent to inhibition of this enzyme (3). To compare these effects of TR on nucleoside triphosphate formation in the sensitive and resistant lines, mice bearing subcutaneous nodules of P388/S and P388/TR were given TR at a dose level of 250 mg/kg, i.p., and killed at the time points illustrated in Fig. 3.

In mice bearing P388/S, GTP concentrations were markedly reduced after treatment with TR and reached a nadir 4-8 hr after TR treatment, as shown in panel A of Fig. 3. In addition, there was a 25% decrease in the concentration of ATP at 4 hr, while there was a marked increase in the concentration of UTP and CTP at 8 hr. In contrast, mice bearing subcutaneous implants of P388/TR showed no significant changes in the concentrations of nucleoside triphosphates following TR treatment (panel B of Fig. 3).

These studies indicate that resistance to TR in this murine tumor is related to impaired formation of the anabolite of the latter compound, TAD, with the result that the alterations in nucleoside triphosphate pool sizes which are seen in the sensitive tumor are not observed in the resistant line. A preliminary account of a similar mode of resistance to TR in CHO

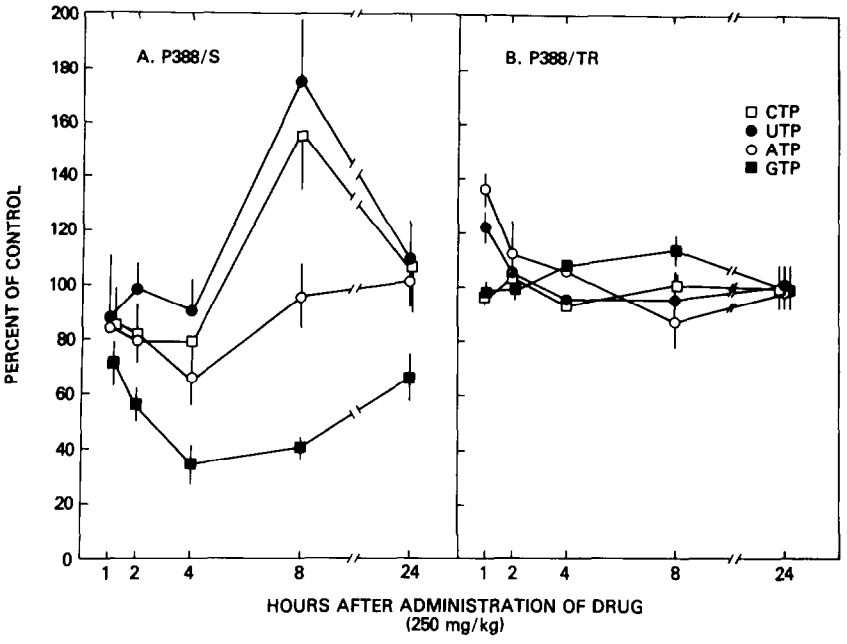

Fig. 3. Groups of five mice bearing subcutaneous P388 tumors were given 250 mg/kg TR, i.p. Tumors were excised at the times indicated, flash-frozen on dry ice, and homogenized in 4 vol. of 5% PCA. The supernatant fractions were neutralized with 40% KOH and aliquots were separated by HPLC by the method previously described (3). Average control nucleotide concentrations (nmoles/g tissue) were: CTP, 157; UTP, 296; ATP, 655; and GTP, 286 for 34 separate determinations.

cells has recently appeared (6). Studies to determine whether this mechanism of resistance is of general applicability to other tumor lines with natural and acquired resistance to TR are now being instituted.

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