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Anticancer Activity of Purine Antagonists and Their Ribosides. I. Comparative Studies with 6-Mercaptopurine and 6-Mercaptopurine Riboside.* (23144)

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Synthesis of 6-mercapto-9-β-D-ribofuranosylpurine (6-MP-riboside) has been achieved in 70-80% yields by reaction of 6-chloro-9-β-D-ribofuranosylpurine with hydrogen sulfide in methanolic sodium methoxide(1). This "fraudulent" riboside was considered worthy of synthesis and trial against experimental neoplasms in view of the well-established antibacterial(2) and antileukemic(3) activity of 6-mercaptopurine (6-MP). The possibility exists that 6-MP may be converted to 6-MP-riboside or ribotide before it becomes an active antimetabolite, and it has been noted that certain 6-MP-resistant bacteria lack the ability to convert hypoxanthine to purine ribotides. To obtain preliminary information on the potential usefulness of 6-MP-riboside, experiments have been carried out to test: (a) Comparative activity of 6-MP and 6-MP-riboside as inhibitors of growth of Adenocarcinoma 755, an experimental neoplasm of unusual sensitivity to known purine antagonists (4). (b) Sensitivity of *Streptococcus faecalis* (ATCC No. 8043, SF/O) and 6-MP-resistant lines of this bacterium to inhibition by 6-MP-riboside. (c) Effects of 6-MP-riboside on growth of a 6-MP-resistant line of Adenocarcinoma 755.

The results obtained in such studies are reported herein.

Inhibition of Adenocarcinoma 755. Highly inbred C57 black mice obtained from Jackson Memorial Laboratories were implanted with Adenocarcinoma 755 by the usual trocar method, randomized, and broken into groups of 10 mice each. The comparative inhibitory effects of 6-MP and 6-MP-riboside on growth of this tumor were then determined. Both compounds were administered intraperitoneally; therapy was initiated 24 hours after tumor implantation and continued once a day (Schedule B) or 3 times daily (Schedule C) for 11 or 12 days. All tumors were excised and weighed individually on the 12th and 14th day. The results of these inhibition assays are summarized in Table I.

Effects of 6-Mercaptopurine and 6-Mercaptopurine Riboside on Growth of Streptococcus faecalis. To learn something of the cross-resistance in biologic systems between 6-MP and its riboside, experiments were carried out in which *S. faecalis* (SF/O) and two 6-MP-resistant lines of *S. faecalis* (SF/MP(5) and SF/MPcc†) were compared as to their susceptibility to growth inhibition by 6-MP and its riboside. A modification of the medium of Flynn, *et al.*(6) was used in these experi-

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† This resistant line was isolated by the same procedure as was SF/MP(5).

TABLE I. Comparative Effects of 6-Mercaptopurine and 6-Mercaptopurine Riboside on Growth of Adenocarcinoma 755.

Exp. No.	Treatment	Dosage mg/kg/day	Dosage mM/kg/day	Avg animal wt change (g)	Mortality (out of 10)	Avg tumor wt, % of control	Dosage schedule
1	6-MP	30	.20	+1	2	2	B
		15	.10	+1	1	3	B
		7.5	.05	+2	1	3	B
	6-MP-riboside	70	.25	+1	0	2	B
		35	.13	+1	0	3	B
		17	.07	+1	0	2	B
2	6-MP	15	.10	−2	4	0	C
		7.5	.05	−1	2	0	C
		3.8	.025	−1	0	0	C
		1.9	.012	0	1	15	C
		.9	.006	+1	0	79	C
		.45	.003	0	0	88	C
		.10	.0006	+1	0	96	C
	6-MP-riboside	17	.07	−3	3	0	C
		8.5	.035	−2	1	0	C
		4.3	.018	−2	1	3	C
		2.1	.009	0	2	47	C
		1.1	.005	+1	1	84	C
		.25	.001	0	0	84	C

Note: Therapy was initiated 24 hr following tumor implantation and continued once daily for 11 days (B schedule) or 3 times daily for 12 days (C schedule). Tumors were weighed on 12th day (B schedule) or 14th day (C schedule).

ments. Adenine, guanine, xanthine, and uracil were omitted from the medium. This purine-pyrimidine-free basal medium was supplemented with pteroylglutamic acid, 3 mγ/ml, and sodium acetate, 100 γ/ml. To avoid exposure of 6-MP or 6-MP-riboside to heat, all solutions and medium were filtered through ultrafine sintered glass filters and added aseptically to sterile tubes. The inoculum for each tube consisted of 0.1 ml of a twice washed culture which contained approximately 1.5 x 10^6 cells. Growth was measured as optical density at 660 millimicrons in a Coleman Spectrophotometer after 22 hours of incubation at 35°C. The results of this study are summarized in Table II.

Effects of 6-MP-Riboside on a 6-MP-Resistant Line of Adenocarcinoma 755. Although microbiologic studies showed that 2 different 6-MP-resistant lines of *S. faecalis* were solidly cross-resistant to 6-MP-riboside, it was

TABLE II. Effects of 6-Mercaptopurine and 6-Mercaptopurine Riboside on Growth of *S. faecalis*.

Inhibitor (γ/ml)	Optical density at 22 hr: 6-MP SF/O	6-MP SF/MP	6-MP SF/MPcc	6-MP-riboside SF/O	6-MP-riboside SF/MP	6-MP-riboside SF/MPcc
500	0	0	0	0	0	.07
150	.10	.47	.23	.04	.53	.29
50	.25	.52	.30	.05	.48	.38
15	.25	.42	.32	0	.48	.30
5	.18	.38	.30	0	.47	.27
1.5	.19	.38	.30	0	.48	.24
.5	.08	.38	.23	0	.48	.25
.15	0	.39	.29	0	.47	.27
.05	0	.38	.29	0	.48	.28
.015	.21	.39	.30	.23	.51	.32
.005	.34	.40	.30	.33	.49	.32
.0015	.34	.40	.29	.35	.49	.32
.0005	.36	.42	.30	.35	.50	.33
0	.34	.49	.36	.36	.47	.31

TABLE III. Effects of 6-Mercaptopurine Riboside on Growth of a 6-MP-Resistant Line of Adenocarcinoma 755 (755/MP).

Exp. No.	Tumor	Treatment	Dosage (mg/kg/day)	Avg animal wt change (g)	Mortality (out of 10)	Avg tumor wt, % of untreated controls
1	755	6-MP	20	− .7	1	1
	755/MP		20	+ .1	0	59
	755		10	− .5	0	5
	755/MP		10	+2.1	0	88
	755		5	+ .1	1	13
	755/MP		5	+3.0	0	96
	755	6-MP-riboside	34	−1.0	0	3
	755/MP		34	+2.5	0	100
	755		17	−1.9	1	1
	755/MP		17	+2.6	0	100
2	755	6-MP	20	− .9	1	.6
	755/MP		20	−3.2	5	34
	755		10	+ .7	0	2
	755/MP		10	− .1	1	46
	755	6-MP-riboside	34	+ .9	0	1
	755/MP		34	−2.4	2	26
	755		17	+ .4	1	2
	755/MP		17	− .5	0	48

Note: Therapy initiated 24 hr after tumor implantation and continued daily for 11 days. Tumors weighed on 12th day.

considered worthwhile to assay this riboside against a 6-MP-resistant neoplasm.

Recently we have selected a 6-MP-resistant line of Adenocarcinoma 755 (755/MP) by serial passage of this tumor in C57 mice treated with 6-MP at levels of 20-40 mg/kg 3 times weekly. After 8 such passages this line of 755 was assayed for response to 6-MP and shown to be significantly resistant.

In additional experiments, the cross-resistance of 755/MP to 6-MP-riboside was studied. Table III.

Discussion. The data presented in Tables I and III clearly indicate that ribosidation of 6-mercaptopurine does not negate the inhibitory activity of this antagonist against Adenocarcinoma 755. Sufficient quantities of 6-mercaptopurine riboside have not yet been available to establish conclusively the relative chemotherapeutic index of this compound for comparison with unsubstituted 6-mercaptopurine; however, the data presented in Table I suggest that the 2 compounds are of the same order of activity. In Experiment No. 2, Table I, the lowest level of 6-MP showing significant activity was 0.012 mM/kg/day (approximately 2 mg/kg/day); 6-MP-riboside significantly inhibited growth of Adenocarcinoma 755 at a level of 0.018 mM/kg/day. These preliminary screening results would not suggest any practical advantage of 6-MP-riboside over 6-MP.

6-Mercaptopurine riboside was synthesized with the hope that it might be inhibitory toward 6-MP-resistant cells. That this hope was not realized may be seen in Table II which shows that 2 different 6-MP-resistant lines of *S. faecalis* are cross-resistant to the riboside and Table III which shows that a 6-MP-resistant line of Adenocarcinoma 755 is cross-resistant to 6-MP-riboside.

The effects of 6-MP and 6-MP-riboside on growth of SF/O show some interesting differences. On a weight basis the 2 compounds have comparable activity with 50% inhibition at 0.03 γ/ml. As concentration of 6-MP was increased to 50 γ/ml growth slightly less than the control occurred, finally with higher concentrations inhibition of growth was again observed. No such growth response was observed with 6-MP-riboside.

It is possible that 6-MP contains a very small amount of hypoxanthine and as the appropriate ratio of purine and antagonist ap-

pears in the medium, growth occurs or growth inhibition is observed. However, chromatographic examination of the 6-MP indicated less than 1% contamination with hypoxanthine. The trough of the response curve disappears after 48 hours incubation, at which time growth in concentrations up to 150 γ/ml is comparable to the control. Inhibition of the riboside is also overcome within a 120-hour incubation period. An alternative explanation for the 6-MP results is that 6-MP over a narrow concentration range serves as a purine source (after conversion to hypoxanthine) and thus stimulates growth and overcomes to a degree unchanged antagonist at certain concentrations. Inhibition by low levels in the early phase of growth may be due in some way to interference with the *de novo* formation of coenzymes or precursors necessary for biosynthesis of nucleic acids.

The present results require considerable extension prior to any final conclusion regarding the possible value of 6-mercaptopurine riboside as a chemotherapeutic agent; however, to date no evidence has been obtained which would suggest any advantage over the much more easily prepared 6-mercaptopurine.

Observation of cross-resistance of 6-mercaptopurine-resistant bacterial and neoplastic cells to 6-MP-riboside deletes one theoretical explanation of the mechanism of 6-MP-resistance—that is, that such resistant cells have lost the ability to ribosidate 6-MP.

Summary. (1) 6-Mercaptopurine riboside has been assayed for inhibitory activity against Adenocarcinoma 755 in mice and has been found to be of approximately the same activity as 6-mercaptopurine. (2) Lines of *S. faecalis* resistant to 6-mercaptopurine have been found to be cross-resistant to 6-mercaptopurine riboside. (3) A line of Adenocarcinoma 755 selected for resistance to 6-mercaptopurine showed cross-resistance to 6-mercaptopurine riboside.

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Influence of Thiourea on Growth of Cells of Midbrain in Frogs.* (23145)

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Recent studies demonstrate that thyroid hormone affects the differentiation of particular cells of the amphibian central nervous system. Following treatment with thyroxine, certain cells of the medulla oblongata grow (1,2). Cells of the lateral motor column increase their nuclear size in response to both direct and indirect thyroxine stimulation(3). Several independent lines of investigation have shown that the level of differentiation attained by cells of the mesencephalic nucleus of the trigeminal nerve in tadpoles of *Rana pipiens* is *directly* dependent upon concentration of thyroid hormone available to those cells(4). From these studies it is inferred that blockage of normal metamorphosis by treatment of tadpoles with thiourea might result in termination of growth of the cells of the mesencephalic V nucleus, and perhaps even in their involution. Moreover, treatment of the metamorphosed frog with

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