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STUDIES OF 6-*N*-HYDROXYLAMINO-9-β-D-RIBOFURANOSYLPURINE IN MOUSE LEUKEMIA*

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Abstract—An adenosine analog, 6-*N*-hydroxylamino-9-β-D-ribofuranosylpurine (HAPR) has shown activity against transplanted mouse leukemias L1210 and P815, and against sublines of these resistant to 6-mercaptopurine, methotrexate, vincristine, and 1-β-D-arabinofuranosyl cytosine (Ara C). Conversely, a line of L1210 resistant to HAPR was still sensitive to 6-mercaptopurine, methotrexate, 5-fluorouracil and Ara C but was cross-resistant to 6-methylmercaptopurine riboside (6MeMPR). Adenosine, given simultaneously, blocked the antileukemic effects of HAPR, but adenine and guanine did not. It is postulated by analogy with 6MeMPR that HAPR may be phosphorylated to an active nucleotide by adenosine kinase, and that HAPR resistance may be due to loss of adenosine kinase activity. The antileukemic effects of HAPR were potentiated by 6-mercaptopurine.

THE ADENINE antagonist, 6-*N*-hydroxylaminopurine (HAP), was synthesized by Giner-Sorolla and Bendich[1] and found to have some selective antitumor activity against sarcoma 180 in tissue culture by Biesele.[2] It was shown to inhibit the growth of Ehrlich ascites carcinoma *in vivo* and also to cause a moderate increase in life span of mice bearing the ascitic form of sarcoma 180 by Sartorelli *et al.*[3,4] In their studies it produced no significant prolongation of life in mice with leukemia L1210, either sensitive to or resistant to 6-mercaptopurine. In the hope of increasing the antitumor activity, the ribosyl derivative, 6-*N*-hydroxylamino-9-β-D-ribofuranosylpurine (HAPR), was synthesized,[5–7] and preliminary studies in our laboratory showed it was active against transplanted mouse leukemias P815 and P388.[7,8] Bloch *et al.*[9] have also recently reported its activity against leukemia L1210 and sarcoma 180 ascites *in vivo*. This compound has now been studied in detail against various sensitive and resistant lines of leukemia and has been compared quantitatively with the parent compound HAP. The results of these studies are herewith reported.

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METHODS

The technic for evaluating the chemotherapeutic activity of a drug by its ability to prolong the survival time of mice with transplanted leukemia has been reported previously.[10] The experiments described here were done with leukemias P815[11] and L1210,[12] and sublines thereof resistant to various conventional chemotherapeutic agents, in F_1 hybrids of the C57BL/6 × DBA/2 cross (BDF). One million leukemic cells in a saline suspension were inoculated i.p. into each animal, which produced an ascitic leukemia which later progressed to a generalized disease. The mice were divided into groups of ten mice each, and treatment was initiated 24 hr after the inoculation with leukemia and continued once daily i.p. to a total of ten doses. All purines and purine ribosides were dissolved in saline or suspended in a 0·5% solution of carboxymethylcellulose in saline. The mice were weighed twice weekly and autopsied at death for gross evidence of leukemia.

RESULTS

As can be seen from Table 1, HAPR was considerably more active against transplanted leukemias L1210 and P815 with less toxicity than HAP, both on a molar and

TABLE 1. EFFECT OF HAPR[a] AND HAP[b] ON SURVIVAL TIME OF MICE WITH LEUKEMIAS L1210 AND P815

Compound	Dose (mg/kg qd × 10)[c]	Weight change (g)[d]	Survival time (days)	ILS[e]
Control (L1210)			9·1	
HAPR[a]	270	+1·7	26·3	189
	180	+1·5	19·6	115
	120	+0·5	20·5	125
HAP[b]	300	−3·2	16·2	78
	200	−0·4	16·9	86
	133	−0·8	15·1	66
Control (P815)		+3·2	8·4	
HAPR	270	−0·1	19·1	127
	180	+0·3	17·5	108
	120	+1·4	14·1	68
HAP	300	−2·4	10·3	23
	200	−3·7	11·2	33
	133	−0·1	12·2	45

[a] 6-*N*-Hydroxylamino-9-β-D-ribofuranosylpurine.
[b] 6-*N*-Hydroxylaminopurine.
[c] Treatment begun 24 hr after injection of leukemia.
[d] Weight change determined at 14 days for first experiment, at 8 days for second experiment.
[e] Increase in life span, per cent.

actual dose basis. Table 2 shows that HAPR was also active against lines of leukemias P815 and L1210 resistant to 6-mercaptopurine. There was a reasonably broad chemotherapeutic range with activity at doses ranging from 60 to 300 mg/kg. HAPR also demonstrated definite activity on lines of leukemia P815 resistant to various other conventional chemotherapeutic agents such as methotrexate, vincristine, actinomycin D, or 1-β-D-arabinofuranosyl cytosine (Ara C) (Table 3). A line of L1210 leukemia which had become resistant to HAPR after repeated passage through mice treated with HAPR (100 mg/kg daily), was still sensitive to 6-mercaptopurine, thioguanine,

methotrexate, Ara C, and 5-fluorouracil, but not to 6-methylmercapto-9-β-D-ribofuranosyl-purine (6MeMPR) (Table 4). The lack of cross-resistance between HAPR and 6-mercaptopurine suggested that these two purine derivatives were working by different mechanisms of action and therefore might be additive or

TABLE 2. EFFECT OF HAPR ON SURVIVAL TIME OF MICE WITH LINES OF LEUKEMIAS P815 AND L1210 MADE RESISTANT TO 6-MERCAPTOPURINE

Compound	Dose (mg/kg qd × 10)	Weight change (g)[a]	Survival time (days)	ILS (%)
Control (P815/MP)		+3·1	8·7	
6-Mercaptopurine	40	+0·2	8·2	−6
	20	+1·3	8·1	−7
	10	+1·0	7·9	−9
HAPR	135	+0·1	16·1	85
	90	+1·6	14·8	70
	60	+1·1	11·1	28
Control (P815/MP)		+3·7	7·9	
HAPR	300	−1·0	30·5	286
	200	+0·2	23·6	199
	90	+1·5	21·4	171
	60	+1·2	21·3	170
Control (L1210/MP)		+1·2	9·3	
6-Mercaptopurine	40	−1·2	8·6	−8
	20	0·0	8·6	−8
HAPR	100	−1·2	19·7	112

[a] Weight change determined at 7 days for first and second experiments, at 6 days for third experiment.

TABLE 3. EFFECT OF HAPR ON SURVIVAL TIME OF MICE WITH SUBLINES OF LEUKEMIA P815 RESISTANT TO VARIOUS COMPOUNDS OF VALUE IN CLINICAL ACUTE LEUKEMIA

Compound	Dose (mg/kg qd × 10)	Weight change (g)[a]	Survival time (days)	ILS (%)
Control (P815/MTX)[b]		+3·5	8·5	
HAPR	300	+0·3	16·0	88
	200	+1·8	15·8	86
	90	+3·1	16·2	91
Control (P815/VCR)[c]		+3·0	9·0	
HAPR	200	+0·9	17·4	93
	90	+1·2	17·0	89
	60	+1·1	16·8	87
Control (P815/Act D)[d]		+2·4	7·6	
HAPR	200	+0·4	15·7	106
	90	+0·5	15·1	99
	60	+1·2	15·0	97
Control (P815/Ara C)[e]		+4·1	9·9	
HAPR	300	−1·1	18·7	89
	200	−0·6	15·6	58
	90	+2·3	15·0	52

[a] Weight change determined at 9 days for first experiment, at 8 days for second and fourth experiments, at 7 days for third experiment.
[b] Resistant to methotrexate.
[c] Resistant to vincristine.
[d] Resistant to actinomycin D.
[e] Resistant to Ara C.

TABLE 4. EFFECT OF VARIOUS COMPOUNDS ON SURVIVAL TIME OF MICE WITH A SUBLINE OF LEUKEMIA L1210 RESISTANT TO HAPR (L1210/HAPR)

Compound	Dose (mg/kg qd × 10)	7-Day weight change (g)	Survival time (days)	ILS (%)
Control		+2·7	11·7	
6-MP	10	+0·3	21·4[a]	83
MTX	4[b]	−0·1	24·2[a]	107
CA	20	−1·2	23·2[a]	98
FU	13	0·0	22·3[a]	91
6-MeMPR	20	−0·9	11·7	0
HAPR	200	+1·5	10·5	−10

[a] Survivors at 30 days.
[b] q2d × 5.

synergistic in their therapuetic effect. Such potentiating effects are shown in Table 5, particularly at low doses (50 mg/kg) of HAPR that produced an increase in life span (ILS) of 109 per cent, and 5 mg of 6-mercaptopurine/kg that showed an increase of 15 per cent, but the combination gave an ILS of 231 per cent. Even at doses

TABLE 5. EFFECTS OF COMBINATION THERAPY OF HAPR AND 6-MERCAPTOPURINE (6MP) ON SURVIVAL TIME OF MICE WITH LEUKEMIAS P815 AND L1210

Compound	Dose (mg/kg qd × 10)	8-Day weight change (g)	Survival time (days)	ILS (%)
Control (P815)		+3·2	8·4	
HAPR	270	−0·1	19·1	127
	180	+0·3	17·5	108
	120	+1·4	14·1	68
6MP	20	+0·1	15·8	88
HAPR +	180			
6MP	20	−1·1	28·4	238
Control (L1210)		+4·6	8·5	
HAPR	200	+1·0	20·1	136
	100	+1·9	15·9	87
	50	+2·9	17·8	109
6MP	20	−1·0	20·6	142
	10	+3·6	11·4	34
	5	+3·7	9·8	15
HAPR +	100			
6MP	10	+0·2	36·2	326
	100			
	5	+0·2	30·4	258
	50			
	10	+0·8	31·5	271
	50			
	5	+1·9	28·1	231

almost fourfold greater (180 and 20 mg/kg respectively), however, there was very little toxicity.

Since Sartorelli *et al.*[4] had shown that the inhibitory effects of HAP could be blocked *in vitro* by adenine, we studied the possible antagonistic relationship of adenine and adenosine *in vivo* to HAPR. Table 6 shows the ability of adenosine at 100–200 mg/kg

to block completely the antileukemic effect of HAPR at 50–200 mg/kg, when both were given i.p. daily for 10 days in mice bearing L1210 leukemia. Adenine and guanine, however, at the doses used, were unable to block this antileukemic effect.

TABLE 6. EFFECTS OF ADENOSINE (ADR), ADENINE (AD) AND GUANINE (GU) IN BLOCKING THE ANTILEUKEMIC EFFECT OF HAPR IN LEUKEMIA L1210

Compound	Dose (mg/kg qd × 10)	Weight change (g)[a]	Survival time (days)	ILS (%)
Control		+4·0	8·5	
HAPR	200	+0·8	17·6	107
HAPR +	200			
AdR	200	+3·8	10·4	22
AdR	200	+4·0	9·0	6
Control		+2·3	8·4	
HAPR	100	0·0	25·1	199
HAPR +	100			
AdR	200	+3·9	8·5	1
HAPR +	50			
AdR	100	+4·5	11·7	39
HAPR +	100			
Ad	50	−0·2	21·6	157
HAPR +	50			
Ad	50	+0·6	24·1	187
HAPR +	100			
Gu	100	−0·5	27·9	232
HAPR +	50			
Gu	50	+0·5	25·9	208
AdR	200	+1·7	8·4	0
	100	+1·9	8·1	−4
Ad	50	+1·5	8·6	2
Gu	100	+1·9	8·3	−1
	50	+2·3	8·0	−5

[a] Weight change determined at 6 days for first experiment, at 8 days for second experiment.

DISCUSSION

HAPR has demonstrated a definitely greater antileukemic activity than HAP for the same degree of toxicity. This lower toxicity may be analogous to the adenosine–adenine differences and may be due to the relatively greater solubility of oxidation products of the ribosyl derivative, and therefore decreased kidney toxicity of HAPR.[13,14] The ability of adenosine, but not of equimolar doses of adenine or guanine, to prevent the antileukemic effects of HAPR in leukemia L1210 suggests that HAPR is acting as an adenosine antagonist. It has previously been reported by Bennett *et al.*[15,16] and Caldwell *et al.*[17] that 6MeMPR is phosphorylated by adenosine kinase and that neoplasms resistant to this agent have lost adenosine kinase activity.[15,16] The fact that both HAPR and 6MeMPR were active against a line of L1210 leukemia resistant to 6-mercaptopurine (6MP) which has lost the pyrophosphorylase that converts 6MP to the active 6MP ribonucleotide,[18] suggests that HAPR also does not reach the active nucleotide level by the pyrophosphorylase pathway. The effectiveness of adenosine but not of adenine in preventing the inhibition of L1210 by HAPR may be attributable to competition for adenosine kinase. These data and the fact that the HAPR-resistant line of leukemia L1210 is also cross-resistant to 6MeMPR but not to 6-mercaptopurine or thioguanine suggests that HAPR is also phosphorylated by

adenosine kinase and that HAPR resistance in L1210/HAPR may be due to loss of this kinase activity. The activity against lines resistant to 6-mercaptopurine and other conventional agents indicates that HAPR might be useful in patients with acute leukemia whose disease has developed resistance to 6-mercaptopurine, vincristine, methotrexate, and Ara C. The additive effects of HAPR and 6-mercaptopurine in mouse leukemias also suggest that this combination might merit clinical trial.

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