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UTILIZATION OF PREFORMED PURINES BY SARCOMA 180 OR HEPATOMA 134 CELLS TREATED WITH 6-CHLOROPURINE OR A COMBINATION OF 6-CHLOROPURINE AND AZASERINE*

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Abstract—The metabolism of preformed purines was compared in sarcoma 180 and hepatoma 134 ascites tumor cells exposed to either 6-chloropurine or a combination of chloropurine and azaserine. Marked alterations in purine utilization occurred in the drug-sensitive cells (sarcoma 180) treated with chloropurine. These were an increased incorporation of adenine and guanine into polynucleotide guanine, an increased conversion of guanine to acid-soluble adenine, and an increased conversion of hypoxanthine to both polynucleotide and acid-soluble adenine. Preformed purine metabolism of hepatoma 134, a neoplasm in which chloropurine did not enhance the growth-retarding properties of azaserine, proved to be relatively refractive to chloropurine. An increased conversion of preformed guanine to acid-soluble adenine occurred as a consequence of chloropurine treatment. Exposure of sarcoma 180 to a combination of chloropurine and azaserine increased the rate of conversion of preformed purines to adenine nucleotides over that found with chloropurine alone. Hepatoma 134 also responded to the drug combination by an increase in the rate of preformed purine conversion to adenine nucleotides. These reactions were interpreted as manifestations of compensatory responses to the stress of metabolic blocks on the pathway of purine synthesis *de novo*.

INTRODUCTION

IN THE previous paper[1] the tumor-inhibitory properties of combinations of 6-chloropurine and azaserine were found to correlate with the blockage of guanine nucleotide biosynthesis *de novo* by each agent in four ascites cell neoplasms. Hepatoma 134 was an exception, for both chloropurine and azaserine produced a decreased rate of guanine formation comparable to that obtained with sensitive tumors, although this neoplasm was not sensitive to the drug combination. Since it was conceivable that the growth-retarding properties of this dual block were minimized in hepatoma 134 by differences in the relative importance of alternate metabolic routes, the ability of this neoplasm to utilize preformed purines was compared with sensitive sarcoma 180 cells during exposure to chloropurine or a combination of chloropurine and azaserine. A preliminary report of this work has been presented.[2]

MATERIALS AND METHODS

Sarcoma 180 ascites cells were maintained in female Ha/ICR Swiss mice (The A. R. Schmidt Co., Madison, Wis.), and hepatoma 134 ascites cells were maintained

* The following abbreviations are used: NA for nucleic acid and AS for acid-soluble.

in female C3H mice (Texas Inbred Mice Co., Houston, Texas). Each tumor was transplanted 6 days before use into mice of the C3H strain. Cells were withdrawn from donor animals, centrifuged for 2 min in a clinical centrifuge (1600 *g*), suspended in 10 vols. of isotonic saline and 0·1 ml of the suspension was injected into each mouse.

Drugs were dissolved in isotonic saline and injected intraperitoneally: azaserine at a dose of 0·2 mg/kg and chloropurine (Nutritional Biochemicals Corp., Cleveland, Ohio) at a level of 40 mg/kg. At various times after the injection of the drug or drugs, each mouse received an intraperitoneal injection of either 50 μg of guanine-8-^{14}C (California Corp. for Biochemical Research, Los Angeles, Calif.) (12×10^3 counts/min per μg), 50 μg of adenine-8-^{14}C (California Corp. for Biochemical Research) (14×10^4 counts/min per μg), or 50 μg of hypoxanthine-8-^{14}C (Nuclear-Chicago Corp., Chicago, Ill.) ($9{\cdot}4 \times 10^3$ counts/min per μg). Guanine-8-^{14}C was allowed to incorporate for 20 min, while adenine-8-^{14}C and hypoxanthine-8-^{14}C were allowed 1 hr. At the end of the incorporation period, the cells were harvested, and nucleic acid purines and acid-soluble adenine were isolated and analyzed as described previously.[1] Each experimental point represents the average of values obtained from separate analyses of ascites cells from four to eight mice.

RESULTS

In earlier reports,[2, 3] inhibition of the formation of guanine *de novo* by chloropurine in sarcoma 180 ascites cells was shown to be accompanied by increased utilization of certain preformed purines. This apparent compensatory mechanism was compared in cells sensitive (sarcoma 180) and resistant (hepatoma 134) to combinations of azaserine and chloropurine by measuring the effect of chloropurine on the rate of guanine-8-^{14}C utilization for purine formation (Table 1). Untreated cells of both tumors appeared to

TABLE 1. EFFECT OF 6-CHLOROPURINE ON GUANINE-8-^{14}C INCORPORATION INTO PURINES OF SARCOMA 180 AND HEPATOMA 134 CELLS

Neoplasm	Hours after chloropurine dose	Counts/min per μmole $\times 10^{-2}$		
		NA guanine	NA adenine	AS adenine
Sarcoma 180	0	20·2±1·7*	1·4±0·2	48·0± 2·8
	1	23·5±3·2	1·3±0·3	67·1± 9·8
	3	35·0±4·9	1·9±0·4	154·8±32·5
Hepatoma 134	0	24·2±1·3	1·1±0·1	92·3±10·7
	1	23·6±2·0	1·1±0·05	215·9±45·9
	3	20·3±0·7	0·9±0·1	153·9±23·8

* Standard deviation of the mean.
Tumor-bearing mice were each injected intraperitoneally with 40 mg chloropurine per kg. At the designated time, 50 μg guanine-8-^{14}C (12×10^3 counts/min per μg) per mouse were injected and were allowed to be incorporated for 20 min. The zero time points represent the results obtained with control mice that received an injection of isotonic saline 1 hr before radioactive guanine.

utilize guanine for polynucleotide formation at similar rates. Following chloropurine treatment, an increased incorporation of isotope into polynucleotide guanine and acid-soluble adenine was obtained in sarcoma 180 cells, while only the conversion of radioactive guanine to acid-soluble adenine was increased in treated hepatoma 134.

The changes in adenine metabolism induced by chloropurine were estimated in these two neoplasms by determining the effect of the purine analog on adenine-8-^{14}C uptake (Table 2). Both tumors utilized preformed adenine. Chloropurine administration caused a stimulation of adenine conversion to guanine nucleotides in sarcoma 180 tumor-bearing mice. No significant alterations in adenine incorporation into purines occurred in drug-treated hepatoma 134 cells.

TABLE 2. EFFECT OF 6-CHLOROPURINE ON ADENINE-8-^{14}C INCORPORATION INTO PURINES OF SARCOMA 180 AND HEPATOMA 134 CELLS

Neoplasm	Hours after chloropurine dose	Counts/min per μmole $\times 10^{-2}$		
		NA guanine	NA adenine	AS adenine
Sarcoma 180	0	4·5±0·3*	78·3±6·3	1290± 54
	1	8·1±1·8	67·9±9·2	1215±116
	3	8·4±1·9	58·9±9·3	1116± 72
Hepatoma 134	0	3·7±0·3	49·3±4·3	759± 72
	1	3·1±0·3	43·5±3·1	713± 28
	3	3·6±0·5	39·2±4·7	733± 66

* Standard deviation of the mean.
Tumor-bearing mice were each injected intraperitoneally with 40 mg chloropurine per kg. At the designated time, 50 μg adenine-8-^{14}C (14×10^4 counts/min per μg) per mouse were injected and were allowed to be incorporated for 1 hr. The zero time points represent the results obtained with control mice that received an injection of isotonic saline 1 hr before radioactive adenine.

TABLE 3. EFFECT OF 6-CHLOROPURINE ON HYPOXANTHINE-8-^{14}C INCORPORATION INTO PURINES OF SARCOMA 180 AND HEPATOMA 134 CELLS

Neoplasm	Hours after chloropurine dose	Counts/min per μmole $\times 10^{-2}$		
		NA guanine	NA adenine	AS adenine
Sarcoma 180	0	12·0±1·1*	15·2±1·0	264·2±13·4
	1	12·6±0·9	29·5±0·8	446·5±13·2
	3	8·8±1·5	18·6±1·4	335·1±15·3
	6	12·3±2·2	18·2±1·4	311·2±15·1
Hepatoma 134	0	4·4±0·6	13·6±1·3	210·4±14·8
	1	2·8±0·2	14·1±0·5	235·5± 6·7
	3	3·2±0·3	12·8±0·7	211·6± 9·4
	6	5·3±0·4	14·6±1·3	189·2± 8·4

* Standard deviation of the mean.
Tumor-bearing mice were each injected intraperitoneally with 40 mg chloropurine per kg. At the designated time, 50 μg hypoxanthine-8-^{14}C ($9{\cdot}4 \times 10^3$ counts/min per μg) per mouse were injected and were allowed to be incorporated for 1 hr. The zero time points represent the results obtained with control mice that received an injection of isotonic saline 1 hr before radioactive hypoxanthine.

Table 3 shows the effects of the drug on hypoxanthine-8-^{14}C incorporation into purines of the two tumors. Hypoxanthine appeared to be utilized for polynucleotide guanine formation at a greater rate in sarcoma 180 cells, while the conversion of this metabolite to adenine compounds was similar in the two cell lines. In sarcoma 180,

chloropurine treatment produced a stimulation of hypoxanthine conversion to adenine compounds while, in contrast, no such effect was obtained with the hepatoma.

In order to estimate the effects of a more intense blockage of the synthesis of purines *de novo*, the action of simultaneous administration of azaserine and chloropurine on preformed purine metabolism was compared in the two neoplasms. The

TABLE 4. EFFECT OF SIMULTANEOUS ADMINISTRATION OF 6-CHLOROPURINE AND AZASERINE ON GUANINE-8-^{14}C INCORPORATION INTO PURINES OF SARCOMA 180 AND HEPATOMA 134 CELLS

Neoplasm	Hours after drugs	Counts/min per μmole $\times 10^{-2}$		
		NA guanine	NA adenine	AS adenine
Sarcoma 180	0	21·8± 5·4*	1·1±0·2	78·9± 7·3
	1	27·7± 3·0	1·5±0·2	145·2±16·0
	3	39·8± 7·1	1·6±0·2	183·1±19·7
Hepatoma 134	0	19·3± 2·7	0·9±0·3	93·6±10·0
	1	34·5±10·5	1·3±0·4	149·0±27·1
	3	20·2± 3·1	0·8±0·04	253·2±44·2

* Standard deviation of the mean.
Tumor-bearing mice were each injected intraperitoneally with 40 mg chloropurine per kg and 0·2 mg azaserine per kg. At the designated time, 50 μg guanine-8-^{14}C (12×10^3 counts/min per μg) per mouse were injected and were allowed to be incorporated for 20 min. The zero time points represent the results obtained with control mice that received an injection of isotonic saline 1 hr before radioactive guanine.

TABLE 5. EFFECT OF SIMULTANEOUS ADMINISTRATION OF 6-CHLOROPURINE AND AZASERINE ON ADENINE-8-^{14}C INCORPORATION INTO PURINES OF SARCOMA 180 AND HEPATOMA 134 CELLS

Neoplasm	Hours after drugs	Counts/min per μmole $\times 10^{-2}$		
		NA guanine	NA adenine	AS adenine
Sarcoma 180	0	4·7±0·4*	58·8± 1·5	970± 36
	1	5·6±0·4	75·2± 5·6	1303± 78
	3	8·0±0·8	70·6± 3·5	1535±102
Hepatoma 134	0	4·4±1·1	48·0± 4·3	800±100
	1	3·6±0·1	58·9± 3·1	1066± 77
	3	4·6±0·9	64·8±10·4	1353±195

* Standard deviation of the mean.
Tumor-bearing mice were each injected intraperitoneally with 40 mg chloropurine per kg and 0·2 mg azaserine per kg. At the designated time, 50 μg adenine-8-^{14}C (14×10^4 counts/min per μg) per mouse were injected and were allowed to be incorporated for 1 hr. The zero time points represent the results obtained with control mice that received an injection of isotonic saline 1 hr before radioactive adenine.

results of experiments measuring the effects of this drug combination on guanine-8-^{14}C utilization are presented in Table 4. The results obtained with sarcoma 180 cells were similar to those obtained with the purine antagonist alone. Treatment of hepatoma 134 with the drug combination resulted in an increased utilization of guanine for acid-soluble adenine formation. Although this conversion appeared to be less than that found with chloropurine treatment at 1 hr, it was greater at 3 hr.

Table 5 shows the effects of the two drugs on adenine-8-^{14}C uptake. In the sarcoma, stimulation of incorporation into both adenine and guanine occurred following exposure to drugs; however, the increased incorporation into polynucleotide guanine at 1 hr was less than that found with chloropurine alone. In hepatoma 134, azaserine and chloropurine appeared to stimulate adenine-^{14}C utilization for both nucleic acid and acid-soluble adenine. No increase in the conversion to guanine nucleotides was noted.

The data in Table 6 show that the conversion of hypoxanthine-8-^{14}C to adenine and guanine in sarcoma 180 treated with the two antimetabolites was similar to that

TABLE 6. EFFECT OF SIMULTANEOUS ADMINISTRATION OF 6-CHLOROPURINE AND AZASERINE ON HYPOXANTHINE-8-^{14}C INCORPORATION INTO PURINES OF SARCOMA 180 AND HEPATOMA 134 CELLS

Neoplasm	Hours after drugs	Counts/min per μmole $\times 10^{-2}$		
		NA guanine	NA adenine	AS adenine
Sarcoma 180	0	17·9±2·3*	18·8±1·7	322·9± 13·9
	1	16·8±1·5	33·5±1·6	707·2± 65·5
	3	13·2±0·7	27·3±0·4	605·7±118
Hepatoma 134	0	5·6±0·5	11·6±1·1	220·8± 18·4
	1	3·3±0·4	22·8±0·5	429·8± 5·1
	3	4·5±0·2	27·3±3·1	432·0± 48·4

* Standard deviation of the mean.
Tumor-bearing mice were each injected intraperitoneally with 40 mg chloropurine per kg and 0·2 mg azaserine per kg. At the designated time, 50 μg hypoxanthine-8-^{14}C ($9{\cdot}4 \times 10^3$ counts/min per μg) per mouse were injected and were allowed to be incorporated for 1 hr. The zero time points represent the results obtained with control mice that received an injection of isotonic saline 1 hr before radioactive hypoxanthine.

obtained with chloropurine treatment, except that incorporation into acid-soluble adenine was greater than that produced by the purine analog alone. Treatment of hepatoma cells with azaserine and chloropurine caused a marked stimulation in the conversion of radioactive hypoxanthine to adenine compounds. The effect on the conversion to guanine was essentially the same as that produced by chloropurine.

DISCUSSION

Differences in the rates of utilization of preformed purines were found in comparisons of sarcoma 180 and hepatoma 134 ascites cells exposed to chloropurine or a combination of chloropurine and azaserine. Treatment of the drug-sensitive cells (sarcoma 180) with chloropurine caused marked alterations in preformed purine metabolism. These were suggestive of cellular compensatory reactions in response to the duress produced by blockage of a relatively important metabolic reaction(s). The changes included an increased incorporation of adenine and guanine into polynucleotide guanine, an increased conversion of hypoxanthine to adenine compounds, and an increased conversion of preformed guanine to acid-soluble adenine. It is of interest to note that the increased rate of guanine-8-^{14}C incorporation into sarcoma 180 polynucleotide guanine, which was found following chloropurine treatment in this study, did not

occur as soon after exposure to the drug as was previously reported.[3] This difference may be a manifestation of tumor growth in different mouse strains, for earlier experiments[3] were carried out using the mouse strain in which the tumor is normally maintained (Ha/ICR Swiss), while the present experiments were carried out in C3H mice.

In contrast to the sarcoma, preformed purine metabolism of hepatoma 134 cells was relatively unaffected by chloropurine treatment; however, exposure to chloropurine did increase the conversion of guanine-8-^{14}C to acid-soluble adenine. The relative refractiveness of preformed purine metabolism of the hepatoma to chloropurine would appear to correlate with the inability of this agent to potentiate the growth-inhibitory properties of azaserine in this neoplasm.

Since chloropurine caused an inhibition of guanine synthesis *de novo* without concurrent inhibition of adenine formation, a combined treatment of azaserine and chloropurine should provide a more complete block of purine biosynthesis *de novo*. This multiple stress would be expected to create a need for adenine, in addition to the apparent increased cellular requirement for guanine observed in chloropurine-treated cells. Measurement of the utilization of preformed purines after exposure to this drug combination suggested that these requirements were indeed created. Sarcoma 180 and hepatoma 134 ascites cells treated with the combination of drugs showed, in general, a greater increase in the rate of conversion of isotopic purines to adenine nucleotides than that found with chloropurine alone. Differences in the response of the two neoplasms to the drug combination appeared to reside in the utilization of precursors for nucleic acid guanine biosynthesis. An increase occurred in the incorporation of adenine and guanine into polynucleotide guanine of sarcoma 180 cells treated with the drugs, while these agents had no significant effect on these reactions in the hepatoma.

Interpretation of the data obtained with chloropurine as compensatory reactions to the imposed stress correlated with the degree of sensitivity of the two neoplasms to therapy with the drug combination. The findings, however, do not appear to explain the inability of chloropurine to enhance the growth-retarding properties of azaserine against hepatoma 134. Indeed, it is probable that inhibition of additional metabolic pathways, hitherto undiscovered, is required for drug enhancement. In the area of purine catabolism, purified milk xanthine oxidase and purified uricase have been indicated as chloropurine-sensitive enzymes by Duggan and Titus.[4] Inhibition of these enzymes *in vivo* might be expected to provide an increased rate of preformed purine conversion to polynucleotides by conservation of substrate molecules. Such an effect may be in part responsible for the results obtained with chloropurine-treated sarcoma 180 cells.

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