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Inhibition of purine synthesis *de novo* in cultured L5178Y cells by methylthioinosine*

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THE ABILITY OF methylthioinosine† (MMPR) to inhibit the growth of various tumor cells has been attributed to the inhibition of *de novo* purine synthesis by the 5′-monophosphate ester of MMPR, methylthioinosinate (MMPR-P). MMPR-P inhibits the first reaction in the pathway of purine nucleotide synthesis *de novo*, which is catalysed by phosphoribosylpyrophosphate amidotransferase.[1,2] Inhibition of purine nucleotide synthesis by MMPR has been demonstrated in mouse tumor cells (Ehrlich ascites carcinoma,[2,3] leukemia L1210[4] and adenocarcinoma 755 cells[5]) and in human cell lines (H. Ep. No. 2[4] and normal fibroblasts[6–8]).

Strong evidence linking the inhibition of purine synthesis to the growth inhibitory effects of MMPR has recently been presented by Shantz *et al.*,[9] who demonstrated, with Ehrlich ascites tumor cells, correlations between the formation of MMPR-P from MMPR, inhibition of purine synthesis, and inhibition of tumor cell proliferation. Also, Bennett and Adamson[5] have shown that hypoxanthine, adenine and 4-amino-5-imidazolecarboxamide reversed antiproliferative effects of MMPR toward cultured cells of adenocarcinoma 755.

This report describes the antiproliferative effects of MMPR toward cultured cells of the L5178Y mouse lymphoma and relates the reversal of such effects by preformed purines to changes in the sizes of cellular ribonucleotide pools.

The L5178Y lymphoma was maintained by passage *in vivo* in male BDF_1 mice (Laboratory Animal Breeding Service, University of Alberta). Cultures of the lymphoma cells were started with cells obtained from ascitic fluids taken from mice 4–5 days after i.p. implantation with 10^7 L5178Y cells. The lymphoma cells were cultured in Fischer's medium (Grand Island Biological Company, Grand Island, N.Y.) containing 10% horse serum, 100 μg/ml of streptomycin and 100 units/ml of penicillin,

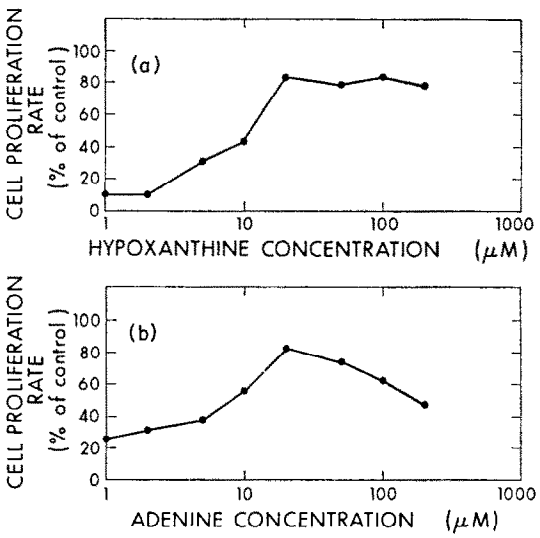

FIG. 1. Reversal by adenine and hypoxanthine of the antiproliferative effects of MMPR. L5178Y cells were cultured in medium containing no test compounds (controls) or 0·5 μM MMPR plus adenine (a), or hypoxanthine (b). Cell proliferation was completely inhibited in the presence of 0·5 μM MMPR. Proliferation rates are expressed as percentages of rates in control cultures.

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† 6-(Methylthio)-9-β-D-ribofuranosylpurine.

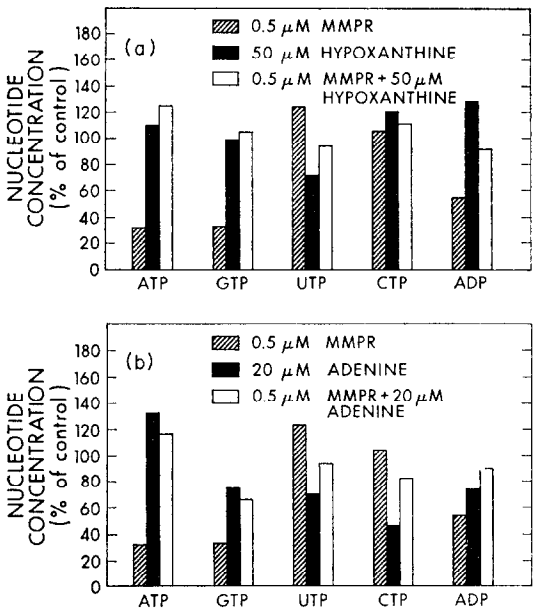

FIG. 2. Nucleotide concentrations in cells protected (by adenine and hypoxanthine) from the antiproliferative effects of MMPR. Cells were cultured for 18 hr in medium containing the following: (a) 0·5 μM MMPR, 50 μM hypoxanthine or 0·5 μM MMPR plus 50 μM hypoxanthine; (b) 0·5 μM MMPR, 20 μM adenine or 0·5 μM MMPR plus 20 μM adenine. Nucleotide concentrations in perchloric acid extracts of the cells were determined by a method involving anion-exchange chromatography, as described earlier.[11] Nucleotide concentrations are expressed as percentages of those found in cells cultured in the absence of test compounds (controls). These control cultures were found to have the following nucleotide concentrations: (a) ATP, 2148 nmoles/10^9 cells; GTP, 712 nmoles/10^9 cells; UTP, 1057 nmoles/10^9 cells; CTP, 674 nmoles/10^9 cells; and ADP, 539 nmoles/10^9 cells; (b) ATP, 2612 nmoles/10^9 cells; GTP, 1006 nmoles/10^9 cells; UTP, 1353 nmoles/10^9 cells; CTP, 1058 nmoles/10^9 cells; and ADP, 776 nmoles/10^9 cells.

as described previously.[10] The ability of purines to reverse the antiproliferative effects of MMPR was evaluated as follows: tests were conducted in duplicate sets of 15 × 85 mm tubes containing initially 4×10^4 cells/ml in 5 ml of culture medium. Cell proliferation was followed for 4-day periods with daily determinations of numbers in 1·0-ml culture samples by means of an electronic particle counter. When cell concentrations reached about 1×10^5 cells/ml, cultures were diluted with medium containing the test compounds to return cell concentrations to initial values. Proliferation rates in test cultures were expressed as percentages of the proliferation rates in control cultures (in which the medium was without test compounds).

Figure 1 shows that adenine or hypoxanthine counteracted the growth inhibitory effects of MMPR toward the lymphoma cells. The culture medium contained 0·5 μM MMPR in both experiments, a concentration which completely inhibited cell proliferation. The presence of hypoxanthine or adenine at concentrations of 20 μM reversed MMPR toxicity to the extent that cells proliferated at about 80 per cent of the control rate. Proliferation rates were not changed by higher concentrations of hypoxanthine, but as adenine concentrations were raised above this level, proliferation rates were progressively reduced, apparently because of adenine toxicity.[5] Guanine did not reverse the antiproliferative effects of MMPR (data not shown).

Concentrations of purine ribonucleotides in L5178Y cells were determined after the cells were incubated for 18 hr in culture medium containing 0·5 μM MMPR plus the following: (a) nothing additional (control), (b) 20 μM adenine, or (c) 50 μM hypoxanthine. Cellular nucleotide concentrations were determined by a method given previously[11] in which perchloric acid extracts of the cultured cells were subjected to high-pressure anion-exchange chromatography using the Varian Aerograph LCS-1000 system. As seen in Fig. 2, incubation with 0·5 μM MMPR caused large perturbations in the nucleotide concentrations of L5178Y cells. In agreement with earlier results,[11] cellular

concentrations of ATP, GTP and ADP were reduced by as much as 70 per cent during incubation with 0·5 μM MMPR. Figure 2 shows that concentrations of UTP increased during incubation with MMPR; this result is consistent with other experiments in which the UTP and CTP content of L5178Y cells increased 3-fold over control values during incubation with MMPR (under conditions which differed somewhat from those presently employed).[11]

Figure 2 shows that when the culture medium contained 50 μM hypoxanthine, concentrations of ATP, GTP, ADP and CTP were equal to or greater than those in control cells, whereas that of UTP was reduced. When the medium contained 20 μM adenine, cellular ATP concentrations were somewhat higher than in control cells, but concentrations of the other four nucleotides were reduced. It is apparent in Fig. 2 that when the culture medium contained both 0·5 μM MMPR and 50 μM hypoxanthine, cellular concentrations of the five nucleotides approximated control values; in particular, the marked reductions in ATP and GTP concentrations which occurred in the presence of MMPR were eliminated when the medium contained both MMPR and hypoxanthine. Similarly, in cells cultured in medium containing both 0·5 μM MMPR and 20 μM adenine, ATP and GTP concentrations were increased about 4- and 2-fold, respectively, over those in cells cultured in the presence of 0·5 μM MMPR. As well, the reductions in the pool sizes of the other three nucleotides caused by MMPR were, for the most part, eliminated by the addition of adenine. The ability of hypoxanthine and adenine to protect L5178Y cells from MMPR antiproliferative effects, and to restore cellular concentrations of purine ribonucleotides in the presence of MMPR, indicates that the antiproliferative effect of the analog stems from its ability to inhibit *de novo* purine biosynthesis.

Adenine reversed the antiproliferative effects of MMPR to the same extent as hypoxanthine; this occurred even though the GTP concentration in adenine-protected cells was reduced to about 65 per cent of the control values. Thus, it would appear that adenine was not converted to guanine nucleotides rapidly enough to restore GTP levels to control values; however, the production of guanine nucleotides under these circumstances was evidently sufficient to support cell proliferation. We have reported elsewhere that proliferation rates of L5178Y cells were not affected by 40 per cent reductions in cellular ATP and GTP concentrations.[11]

The inability of hypoxanthine or adenine to reverse completely the antiproliferative effects of MMPR suggests that inhibitory effects of MMPR (or metabolites thereof) at sites other than purine nucleotide synthesis *de novo* may be significant under these circumstances. In this connection, it has been shown that MMPR inhibited incorporation of purine bases into nucleic acids[12] and breakdown of 6-thioguanosine 5′-monophosphate.[13] The present results suggest that purine synthesis *de novo* is the primary site of action of MMPR in L5178Y cells proliferating in culture.

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