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Metabolism of 6-methylthiopurine ribonucleoside 5′-phosphate*

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6-METHYLTHIOPURINE ribonucleoside (NSC 40774, MMPR) is of interest as an antitumor agent, because it is active against certain experimental tumors refractory to treatment with 6-mercaptopurine (NSC 755, MP),[1] and is synergistic in combination with MP in experimental systems.[2,3] MMPR is converted to its nucleotide by adenosine kinase,[4,5] and tumors resistant to MMPR have markedly reduced adenosine kinase activity.[4,6] It is well known that phosphorylated compounds are dephosphorylated in serum and at cell surfaces, after which the components may enter separately into the intracellular fluid.[7–9] This communication describes the dephosphorylation of MMPR-5′-phosphate (MMPR-P) to MMPR in a similar manner. An attempt was also made to use MMPR-P as an antitumor agent, since it might slowly release MMPR, and thus act as a reservoir of MMPR.

MMPR, MMPR-^{35}S and MMPR-P-^{35}S were supplied by the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md. MMPR-P was a gift from Dr. John A. Montgomery, Southern Research Institute, Birmingham, Ala. The chemical purity of the drugs was 96 per cent as determined by chromatographic and radiochemical techniques. Ehrlich ascites carcinoma (EAC) lines sensitive and resistant to MMPR (EAC-R2) were obtained from Dr. A. R. P. Paterson at the University of Alberta, Cancer Research Unit, Edmonton, Alberta, Canada. The tumor lines were maintained by weekly passage in HA/ICR mice.[6] The mice bearing EAC-R2 were maintained on MMPR,[6] except for the generation which was used for the experiment.

Procedures for tissue homogenization, identification of metabolites and for the assay of phosphohydrolase (pH 9·5) have been reported elsewhere.[10,11]

Protein concentrations were determined by the method of Lowry *et al.*[12] using bovine serum albumin as a standard.

TABLE 1. MEASUREMENT OF ADENOSINE KINASE, PHOSPHOHYDROLASE ACTIVITIES AND MMPR-P UPTAKE IN EHRLICH ASCITES CELLS SENSITIVE (EAC) AND RESISTANT TO MMPR (EAC-R2) *IN VITRO*

	EAC		EAC-R2	
Specific activity*	Cells	Ascites fluid	Cells	Ascites fluid
Phosphohydrolase	1·7	3·5	0·4	1·4
Adenosine kinase	9·8	0	0·5	0
Distribution of MMPR-P as % of dose				
MMPR	12	58	5	35
MMPR-P	26	3	0	60

*Specific activity is expressed as nanomoles per milligram of protein per minute by cells or nanomoles per milliliter of fluid per minute. Cells were taken from mice bearing 5-day-old tumors and incubated for 30 min at 37° with 0·29 mM MMPR-P-^{35}S (sp.act., 1×10^5 counts/min/μmole) each in its own ascites fluid.

The uptake and metabolism of MMPR-P were studied by incubating MMPR-P with EAC and EAC-R2 cells in their own ascites fluid (Table 1). After 30 min of incubation, two major products, MMPR-P and MMPR, were found in the EAC cells, while only MMPR was found in EAC-R2 cells. The ascites fluid and cells from both cell lines contained a significant amount of phosphohydrolase activity (Table 1) and, since phosphorylated compounds do not readily cross cell membranes, it appeared that MMPR-P was dephosphorylated to MMPR and then rephosphorylated intracellularly. The incubation of the nucleotide with washed cells in a protein-free Krebs–Ringer medium affected the dephosphorylation at a rate comparable to the incubation of the cells in the ascites fluid. The phosphatase activity of intact cells of *Escherichia coli* in protein-free media has been reported.[9] The

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location of the phosphatase at the cell surface was reported for *E. coli*,[9] EAC cells,[8] and rat liver cells.[7] This study also suggests that MMPR-P was dephosphorylated to MMPR before (in ascites fluid) and/or during entry into cells, and then was rephosphorylated by the intracellular adenosine kinase. Cell lines resistant to MMPR, derived from a human epidermoid carcinoma and from EAC, have negligible levels of adenosine kinase.[4,6] This is further evidenced by the finding that no MMPR-P appeared in EAC-R2 cells after incubating them with the nucleotide (Table 1).

The survival of mice bearing EAC cells treated daily with either MMPR or MMPR-P (500 μmoles/kg) was identical. Mice bearing EAC-R2 cells did not respond to MMPR-P. Similar findings were also reported for arabinosyl cytosine monophosphate (ara-CMP) in leukemia L1210 and its subline resistant to ara-C.[13] This indicated that ara-CMP did not enter cells intact but was dephosphorylated prior to uptake.[13] The mouse survival data suggest that MMPR-P was dephosphorylated at an unfavorable rate, which offers no therapeutic advantage over MMPR. Therefore, the comparison of the rate of MMPR-P excretion with that of MMPR was not pursued.

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Amphetamine–tetrazolium reductase activity in brain

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The observation of Pugh and Quastel[1] that mescaline and amphetamine are attacked either feebly or not at all by brain tissue was confirmed by Bernheim and Bernheim[2] who demonstrated that rabbit liver oxidises mescaline very rapidly. Recently Seiler[3] showed that freshly prepared brain homogenate when incubated with mescaline sulfate at 37° for 18 hr catalysed the oxidation of mescaline by an enzyme which according to him is not diamine oxidase (DAO) but monoamine oxidase (MAO). Axelrod[4–7] and others[8,9] described deamination and hydroxylation of amphetamine, ephedrine and related compounds by liver tissues of various species, but to our knowledge it has not yet been demonstrated that brain tissue can metabolise amphetamine and ephedrine. Although liver possesses an active mescaline oxidase, there is much confusion regarding its nature. Various workers[10,11] suggested that it is different from MAO while Sourkes[12] postulated that mescaline oxidase and DAO are identical. Zeller *et al.*[13] and others[14,15] believe that mescaline is oxidised by MAO, DAO or both. Data are presented in this communication demonstrating the reduction of neo-tetrazolium chloride