Biochemical Pharmacology. Vol. 29 pp. 2261-2264.
Pergamon Press Ltd. 1980. Printed in Great Britain.

PRELIMINARY COMMUNICATIONS

INHIBITION OF INOSINATE DEHYDROGENASE BY 6-AZAURIDINE

Darel Hunting, George Zombor and J. Frank Henderson

Cancer Research Unit and Department of Biochemistry,
University of Alberta,
Edmonton, Alberta, Canada T6G 2H7

(Received 11 May 1980; accepted 30 May 1980)

The growth inhibitory effects of the pyrimidine nucleoside analog, 6-azauridine have been ascribed primarily to inhibition of orotidylate decarboxylase by azauridine 5'-phosphate (reviews: refs. 1,2). As a result of this block, concentrations of pyrimidine nucleotides decline, and other secondary alterations in pyrimidine metabolism may occur. Though most attention has been focused on these effects of azauridine on pyrimidine metabolism, some effects on purine metabolism have also been reported. These include accumulation of hypoxanthine in Escherichia coli treated with azauracil or azauridine (3,4), altered concentrations of adenosine triphosphate (ATP), guanosine triphosphate (GTP) and inosinate in cultured murine lymphoma L5178Y cells treated with azauridine (5), and increased urinary excretion of uric acid in normal and leukemic humans given azauridine (6).

The basis of the effects of azauridine on purine metabolism has not previously been investigated. Here we provide evidence that inosinate dehydrogenase, an enzyme of purine ribonucleotide interconversion, is inhibited in animal cells treated with azauridine.

6-Azauridine was obtained from Sigma Chemical Co. and dissolved in 0.154 M sodium chloride. Sources of other materials, as well as procedures for the thin-layer chromatographic separation of purine bases, ribonucleosides and ribonucleotides and measurement of their radioactivity, have been reported previously (7,8). Nucleotide concentrations were determined using HPLC (9).

Two biological systems were used in these studies. Murine Ehrlich ascites tumor cells were incubated as 2% (v/v) suspensions for 30 min in Fischer's medium containing 5.5 mM glucose, and Chinese hamster ovary (CHO) cells were grown in Alpha Minimal Essential Medium containing 10% dialyzed fetal calf serum, or incubated for 30 min in the same medium.

Table 1. Effects of azauridine on ribonucleotide concentrations in CHO cells*

Azauridine concn. (μM)	Time (hr.)	Growth Rate (percent of control)	Nucleotide Concentrations (percent of control) UTP	CTP	GTP	ATP
0.1	6	89	36	29	79	97
0.3	12	86	25	15	73	120
0.5	13	ND	16	3	60	123
1.0	13	74	11	2	52	128
5.0	13	55	8	1	47	135

*Initial cell density, 100,000 to 200,000 cells/ml.
Control values (nmoles/10^6 cells): UTP, 2.39; CTP, 1.23; GTP, 1.07; ATP, 5.58.

Table 1 shows that while azauridine treatment of CHO cells produced the expected decreases in concentrations of uridine triphosphate (UPT) and cytidine triphosphate (CTP), the concentration of GTP also decreased, though not to the same extent; the concentration of ATP was elevated slightly. At the drug concentrations and treatment times used, growth was inhibited slightly or moderately. These results would be compatible with inhibition of one or both of the reactions leading from inosinate to guanylate in the pathway of GTP synthesis de novo. As the medium used contains no purines, alternative pathways of nucleotide synthesis do not have to be taken into consideration. Furthermore, the fact that ATP concentrations did not decrease suggests that the pathway of inosinate synthesis de novo was not inhibited.

Table 2. Effects of azauridine on purine nucleotide synthesis from [^{14}C]hypoxanthine in CHO cells*

Azauridine concn. (μM)	Nucleotide Radioactivity (percent of control)		
	Guanine nucleotides	Inosinate	Adenine nucleotides
0	100	100	100
0.3	82.9	101	112
0.5	78.5	126	113
2.0	57.2	415	113
5.0	44.7	1476	116

*Initial cell density, 510,000 cells/ml. The final concentration of [^{14}C]hypoxanthine (53 mCi/mmole) was 100 μM. Control radioactivity (counts per min.): guanine nucleotides, 15,960; inosinate, 1,500; adenine nucleotides, 62,120.

To examine the effects of azauridine treatment on the individual reactions of purine ribonucleotide metabolism in more detail, CHO cells were incubated with radioactive hypoxanthine, which is converted to inosinate and thence to both ATP and GTP. As shown in Table 2, the incorporation of hypoxanthine into guanine nucleotides decreased progressively with the concentration of azauridine used; the extents of inhibition observed were similar to the decreases in GTP concentrations reported in Table 1. Concentrations of radioactive xanthylate were very low, and were not affected by azauridine treatment (not shown). Inosinate, however, accumulated to a very considerable degree, and in a dose-related manner in azauridine-treated cells. These results suggest strongly that azauridine was producing its effects on purine metabolism by inhibition of inosinate dehydrogenase rather than by an effect on xanthylate aminase. The observed increase in incorporation of radioactive hypoxanthine into adenine nucleotides presumably was due to a limited redistribution of the inosinate which was not used for guanine nucleotide synthesis.

The accumulation of either inosinate or xanthylate because of drug action would be expected (10) to result in the dephosphorylation of part or all of these nucleoside monophosphates to inosine and xanthosine, respectively; thus analysis of radioactivity in these nucleosides would help provide evidence for the conclusions reached so far. This is technically difficult in the CHO system, however, because of the cell density used and the large volume of medium that would have to be analyzed. For this reason further experiments were conducted using Ehrlich ascites tumor cells incubated at higher cell densities.

Table 3 shows that the incorporation of radioactive hypoxanthine into GTP was inhibited to approximately the same extent in azauridine-treated

Table 3. Effects of azauridine on purine nucleotide metabolism in Ehrlich ascites tumor cells*

Azauridine concn. (μM)	Incorporation of [^{14}C]hypoxanthine (percent of control)			
	GTP	Inosinate	Inosine	ATP
0	100	100	100	100
2.5	48.7	136	104	143
5.0	31.3	159	112	145
7.5	24.5	165	118	150
12.5	20.6	190	132	171

*The final concentration of [^{14}C]hypoxanthine was 100 μM. Control radioactivity (counts per min.): GTP, 10,430; inosinate, 4,740; inosine, 31,430; ATP, 114,430.

Ehrlich ascites tumor cells as in CHO cells. Inosinate also accumulated in a dose-related manner, and a progressive increase in inosine was also observed. (In these cells inosine is formed almost exclusively by the dephosphorylation of inosinate, rather than from hypoxanthine (11).) The concentrations of xanthylate, xanthosine and xanthine remained very low at all azauridine concentrations, again providing evidence that azauridine treatment results in inhibition of inosinate dehydrogenase. Increased incorporation of radioactivity into ATP presumably was due to redistribution of inosinate; it would appear that excess inosinate is used more extensively for ATP synthesis in Ehrlich ascites tumor cells than in CHO cells.

Experiments using pyrazofurin, another inhibitor of orotidylate decarboxylase (12), showed no evidence of inhibition of inosinate dehydrogenase. Thus the effects observed here are specific to azauridine, and not simply secondary to inhibition of the target enzyme or to reduced concentrations of UTP and CTP.

Clearly, several questions remain to be investigated. First, is the effect on inosinate dehydrogenase observed here due to azauridine itself or to azauridine 5'-phosphate? Second, to what extent does inhibition of inosinate dehydrogenase contribute to azauridine-induced growth inhibition and cytotoxicity, in view of more potent effects on orotidylate decarboxylase? Finally, is the effect of azauridine (or its metabolites) direct or indirect? In this case, a direct effect is suggested by the structural resemblance of azauridine to several other heterocyclic inhibitors of inosinate dehydrogenase, including virazole (1-β-D-ribofuranosyl-1,2,4-triazine-3-carboxamide) (13), bredinin (4-carbamoyl-1-ribofuranosyl-imidazolium-5-olate) (14), and 2-amino-1,3,4-thiadiazole (15); however, this point requires further study.

Acknowledgement - This work was supported by the National Cancer Institute of Canada.

References

1. R.E. Handschumacher and A.D. Welch, in E. Chargaff and J.N. Davidson (eds.), The Nucleic Acids, New York, Academic Press 3, 453 (1960).

2. J. Škoda, in A.C. Sartorelli and D.G. Johns (eds.), Antineoplastic and Immunosuppresive Agents, Berlin, Springer-Verlag, Part II, 348 (1975).

3. J. Škoda and F. Šorm, Biochim. Biophys. Acta 28, 659 (1958).

4. J. Škoda and F. Šorm, Coll. Czech. Chem. Commun. 24, 1331 (1959).

5. C.M. Janeway and S. Cha, Cancer Res. 37, 4382 (1977).

6. H.J. Fallon, E. Frei III, J. Block and J.E. Seegmiller, J. Clin. Invest. 40, 1906 (1961).

7. G.W. Crabtree and J.F. Henderson, Cancer Res. 31, 985 (1971).

8. F.F. Snyder and J.F. Henderson, J. Cell. Physiol. 82, 349 (1974).

9. F.F. Snyder, J.F. Henderson, S.C. Kim, A.R.P. Paterson and L.W. Brox, Cancer Res. 33, 2425 (1973).

10. C.A. Lomax, A.S. Bagnara and J.F. Henderson, Can. J. Biochem. 53, 231 (1975).

11. J. Barankiewicz and J.F. Henderson, Biochim. Biophys. Acta 479, 371 (1977).

12. E.C. Cadman, D.E. Dix and R.E. Handschumacher, Cancer Res. 38, 682 (1978).

13. J.K. Lowe, L. Brox and J.F. Henderson, Cancer Res. 37, 736 (1977).

14. K. Sakaguchi, M. Tsujino, M. Yoshizawa, K. Mizino and K. Hayano, Cancer Res. 35, 1643 (1975).

15. J.A. Nelson, L.M. Rose and L.L. Bennett, Jr., Cancer Res. 36, 1375 (1976).