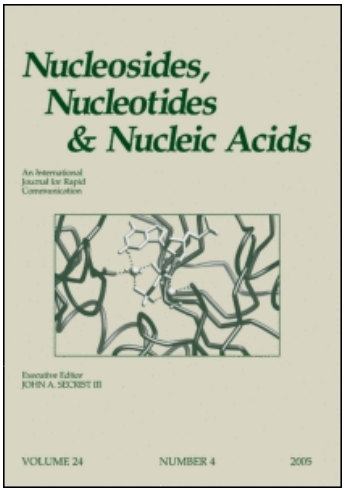

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NUCLEOSIDE AND NUCLEOTIDE INHIBITORS OF INOSINE MONOPHOSPHATE (IMP) DEHYDROGENASE AS POTENTIAL ANTITUMOR INHIBITORS

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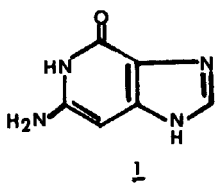
A review of various nucleoside and nucleotide inhibitors of IMP dehydrogenase suggests that such inhibitors may also exhibit significant antitumor effects. The details of the precise mode of action and structure activity relationships remain to be established. This new area of research should prove fruitful for uncovering useful and more specific antitumor agents.

The biochemical conversion of IMP to XMP is catalyzed by the enzyme IMP dehydrogenase. In a study of purine metabolism in tumor versus normal tissue, Weber[1] found that in the rapidly growing hepatoma 3683-F, the level of inosine 5'-monophosphate (IMP) dehydrogenase activity is 10 to 14 times that found in normal rat liver. IMP dehydrogenase increases in parallel with the increase in the growth rate of the tumor. This pattern of enzymatic imbalance increases the tumor cell's ability to synthesize purine nucleotides. Jackson, Morris and Weber[2] have suggested that IMP dehydrogenase is a key enzyme in neoplasia and a most sensitive target for cancer chemotherapy. These authors conclude[2] that IMP dehydrogenase is one of the key rate controlling enzymes of nucleic acid biosynthesis and that the activity of IMP dehydrogenase shows a correlation with cell proliferation in both normal and malignant cells, and that there is a disproportionate increase found in malignant cells of rat liver and kidney which appears to be linked with neoplastic transformation.[2] These authors[3,4] have pointed out that in the rapidly growing hepatoma 3924A and 9618A the specific activity of IMP dehydrogenase increased 1,143% and 1,060% respectively over that found in comparable normal liver. This increase of IMP dehydrogenase in hepatoma links

alteration of the level of activity of this enzyme with neoplastic transformation.[3] The catabolic enzymes of IMP were suppressed in malignant tissue. This alteration of the key enzymes of IMP synthesis, degradation and utilization would appear to be specific to malignancy.[3] These authors have shown that in rat hepatomas and kidney tumors there is an increase in the potential for channelling adenine nucleotides into GTP biosynthesis.[5] Becher and Lohr[6] have studied IMP dehydrogenase and from normal and leukemic leukocytes and have similarly noted that IMP dehydrogenase from leukemic cells had a higher mean specific activity than that from normal leukocytes.

1. Guanosine and Guanylic Acid Analogs

In an effort aimed at designing inhibitors of IMP dehydrogenase, Robins and co-workers,[7,8] synthesized 3-deazaguanine, 1, and the corresponding nucleoside, 2, and 5'-nucleotide, 3, since guanylic acid itself is known to be a natural inhibitor of IMP dehydrogenase.[9] 3-Deazaguanine, 1, 3-deazaguanosine, 2, and 3-deazaguanylic acid, 3, all inhibited IMP dehydrogenase isolated from Ehrlich ascites tumor cells.[10] The active form of the drug is believed to be the 5'-phosphate since this was the most potent inhibitor.[10] 3-Deazaguanine, 1, has been reported by our laboratory[11] to inhibit L-1210 and adenocarcinoma 755 in mice and R320AC mammary adenocarcinoma in rats.



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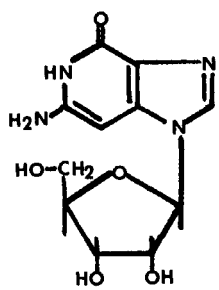

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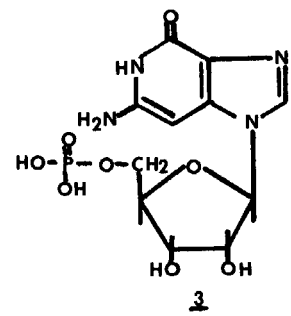
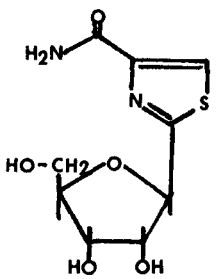


3

3-Deazaguanine is also active against a wide variety of other solid tumors such as mammary adenocarcinoma 13762, C3H mammary adenocarcinoma and colon adenocarcinoma 38 in mice.[11] This activity against L-1210 leukemia has recently been confirmed by the National Cancer Institute for 3-deazaguanine and 3-deazaguanylic acid. Potter[12] has found 3-deazaguanine to be very active against solid tumors in hamsters induced by SV40 transformed cells. 3-Deazaguanine is presently under Phase I

study against human solid tumors in clinical trials. Miller and Adamczyk[13] have recently shown in vitro that six guanosine monophosphate (GMP) analogs were competitive inhibitors of IMP dehydrogenase with respect to IMP. Animal cell IMP dehydrogenase is known to be inhibited by GMP.[14]

Testing data from the National Cancer Institute on 2-β-D-ribofuranosylthiazole-4-carboxamide[15] 4 has recently been made available to us. Against L-1210 in mice, 4 exhibited a T/C of 230% in prolonging survival time of leukemic mice. 2-β-D-Ribofuranosylthiazole-4-carboxamide, 4, exhibits a T/C of 245% against P-388 and is curative at several dose levels against i.v. implanted murine Lewis Lung carcinoma.[16] The synthesis of 4 has been described by Srivastava, Robins and co-workers.[16] The nucleoside, 4, shows a 69% inhibition of the synthesis of guanine nucleotides in Ehrlich ascites tumor cells.[16] Streeter and Miller[17] have recently shown that in cultures of Ehrlich ascites tumor cells 10 μM of 4 gives a 50-60% inhibition of IMP dehydrogenase similar to ribavirin, 6. It has recently been shown[18] that 4 caused a 15-fold increase in IMP concentration in P388 murine leukemia cells. At 8.2 mM, 4 inhibits a crude preparation of IMP dehydrogenase isolated from cultured P388 cells by 50%. The 5'-phosphate of 4, however, was 20 times more potent than 4 in inhibiting the same enzyme.[18] Bennett and co-workers at Southern Research Institute have recently shown that the antitumor activity of 2-amino-1,3,4-thiadiazole is due to the in vitro[19] and in vivo[20] transformation of this compound to a nucleotide (probable structure, 5) which is an excellent inhibitor of an IMP dehydrogenase obtained from L-1210 cells. 2-Amino-1,3,4-thiadiazole lowers guanine nucleotides and elevates IMP. The antitumor activity of 2-amino-1,3,4-thiadiazole has recently been reviewed.[21]



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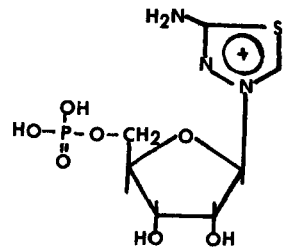
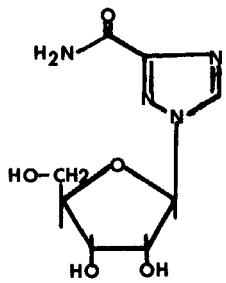


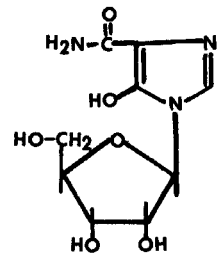
5

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin)[22] 6, has been shown as the 5'-phosphate, to be an inhibitor of IMP dehydro-



Ribavirin

6



Bredinin

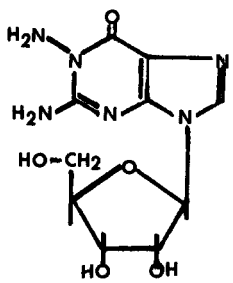
7

genase isolated from Ehrlich ascites tumor cells (K_i=2.5x10^{-7}M).[23,24] Ribavirin is also an active antitumor agent against a number of animal tumors[25] such as L-1210 and adenocarcinoma 755. Potter and co-workers[26,27] have recently shown that 6 greatly suppressed the development of transplanted adenovirus-12 induced tumors in CBA mice. Ribavirin has been shown to be effective against spontaneous AKR leukemia in mice.[28,29] Ribavirin which is converted to the 5'-phosphate by adenosine kinase[30,31] drastically reduces the pool sizes of dGTP and GTP in L-5178Y cells.[32] It has been shown that in cultured lymphoma L-5178Y cells ribavirin, 6, produced a two-fold increase in UTP and CTP with a depletion of GTP and no change in ATP.[33] This expansion of the pyrimidine pools can be explained by the finding that GTP acts as an allosteric inhibitor of glutamine-dependent carbamoyl phosphate synthetase which is the key enzyme in the control of de novo pyrimidine synthesis.[34]

In AS-30D hepatoma cells ribavirin, 6, inhibited IMP dehydrogenase and reduced the soluble guanine nucleotide to 40% of the control. Induction of GTP deficiency was associated with a 50% rise in UTP. Guanosine reversed these effects.[33] It is of considerable interest that the 2'-O-methyl or 3'-O-methyl derivatives of ribavirin-5'-phosphate are inactive as inhibitors of IMP dehydrogenase.[35] Ribavirin is currently under clinical study as an anticancer agent at the Cancer Research Center of the USSR Academy of Medical Science, Moscow.

Closely related in structure to ribavirin is the nucleoside antibiotic Bredinin,[36-39] 7, which inhibits leukemia L-5178Y cells.[36,38,39] Bredinin and bredinin 5'-phosphate[40] are active against L-1210 in the mouse.[36,40] Like ribavirin, the effect of bredinin is reversed by GMP.

The mechanism of action of bredinin has recently been summarized as "inhibiting the conversion of IMP to GMP in the purine biosynthetic pathway.[39] Thus, Bredinin, 7, would seem to be another potential inhibitor of IMP metabolism.



1,2-Diaminoinosine
(1-Aminoguanosine)

8

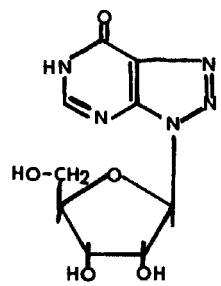
Henderson and co-workers[24] studied the inhibition of IMP dehydrogenase by ribavirin, mycophenolic acid and 1-aminoguanosine, 8, and found these compounds to exert 50% inhibition at 500, 50, and 0.1 μM, respectively. Mycophenolic acid has been reported to inhibit a variety of solid mouse and rat tumors.[41,42] On the basis of these data, they tested 1-aminoguanosine against L-1210 cells in culture and found it to be 50% inhibitory at 400 μM. Thus, the discovery of cytotoxic activity of 1-aminoguanosine was made on the basis of the observation of its activity against IMP dehydrogenase. Henderson and co-workers[24] in the same publication stated: "IMP dehydrogenase occupies a key position in purine metabolism, and potent specific inhibitors of it might be useful chemotherapeutic agents."

In a more recent work, Henderson et al.[34] studied ribavirin and mycophenolic acid in greater detail to see why these inhibitors of IMP dehydrogenase also inhibited cellular growth. It had previously been shown[23] that the inhibition by 6 could be reversed by guanosine or guanylic acid. Henderson et al.[34] found that the effect of mycophenolic acid could also be prevented by the addition of guanylic acid to the medium. It was concluded that the inhibition of IMP dehydrogenase in the L-5178Y cells by either ribavirin or mycophenolic acid was sufficient to result in a decreased concentration of guanine nucleotides which would severely inhibit DNA synthesis. In a recent study of the mechanism of antitumor action of mycophenolic acid, Sweeney[43] noted that

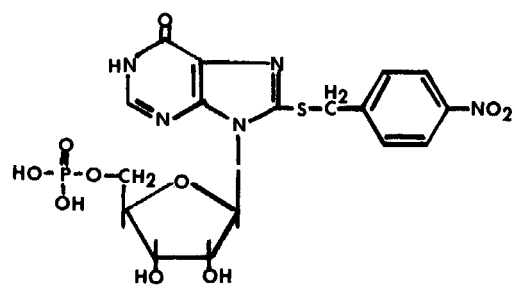
the in vivo antitumor activity against Walker 256 carcinoma in rats could be correlated with a direct inhibition of IMP dehydrogenase.

2. Analogs of Inosine. In view of the fact that inosine 5'-monophosphate (IMP) is, of course, the natural substrate for IMP dehydrogenase, attention has been focused on preparing substrate analogs of IMP which will bind in a tighter fashion to the enzyme. The substrate specificity of IMP dehydrogenase isolated from Sarcoma 180 ascites tumor cells has recently been studied.[13] 8-Aza-IMP, 6-thio-IMP, ara-HxMP and d-IMP were all substrates for IMP dehydrogenases.[13] Perhaps the best type of inhibitor in this category is an IMP analog which would bind tightly but would not be a substrate for the enzymatic reaction. As early as 1963, Hampton[43] showed that the 5'-nucleotides of 6-chloro-, 6-mercapto- and 2-amino-6-mercaptopurine occupied the site of IMP dehydrogenase isolated from Aerobacter Aerogenes. Hampton[44] speculated that such inhibition might indeed be responsible for the antileukemic activity of these drugs. One of the best examples of an inosine analog possessing antitumor activity is 8-azainosine, 9, which was prepared in our laboratory[45] and shown to be active against adenocarcinoma 755 and Leukemia L-1210 in mice. This activity was confirmed at Southern Research Institute.[46] There is evidence that 9 is incorporated into polynucleotides as 8-aza-GMP.[47] Since 8-aza-IMP is a substrate (one-third as active as IMP) for IMP dehydrogenase,[13] it is not clear precisely how 9 may be acting, although the data would indicate that there is a general interference with guanine nucleotide metabolism.

Skibo and Meyer[48] have recently shown that 8-p-nitrobenzylthio IMP, 10, is a potent inhibitor of IMP dehydrogenase isolated from E. coli.



9
8-Azainosine



10

The nucleotide 10 is not a substrate for the enzyme and binds seven-fold stronger to E. coli IMP dehydrogenase than IMP itself.[48] GMP has re-

cently been found to be a competitive inhibitor with respect to IMP dehydrogenase from E. coli.[49] Becher and Lohr[6] have recently noted that IMP dehydrogenase from human leukemic blood cells had a higher mean specific activity than leucocytes from normal patients. These investigators[6] point out that IMP dehydrogenase is an important target enzyme for the 5'-nucleotides of 6-mercaptopurine, 6-thioguanine and 6-chloropurine and that all three purine derivatives show clinical utility in the treatment of childhood leukemia. Indeed, Sartorelli[50] and Hampton[51] have shown 6-chloropurine ribonucleotide[50] to be a good inhibitor of IMP dehydrogenase. Balis[52] and Hampton[51] have shown 6-mercaptopurine-5'-ribonucleotide to exert a similar effect.

The reader should be cautioned, however, that often a nucleoside or nucleotide which is a good inhibitor of IMP dehydrogenase may also have additional sites of action as well. It is of interest that although ribavirin-5'-phosphate and the related nucleotide 2-β-D-ribofuranosylthiazole-4-carboxamide-5'-phosphate are both good inhibitors of IMP dehydrogenase, ribavirin, 6, exhibits good antiviral activity in vivo whereas the nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide, 4, is inactive as an antiviral agent in vivo.[15] Indeed, 4 would, on the other hand, appear to be superior to 6 as an in vivo antitumor agent, since ribavirin is inactive against Lewis lung carcinoma in mice.

In summary, the postulation of Weber[1] that "IMP dehydrogenase is a key enzyme in neoplasia and therefore a sensitive target for cancer chemotherapy" has received considerable experimental support. It would appear that the search for potential inhibitors of IMP dehydrogenase will continue to be a fruitful area for the design and synthesis of potent antitumor agents.

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