Biological Action of Inosine Analogs in Leishmania and Trypanosoma spp.

J. JOSEPH MARR,1,* RANDOLPH L. BERENS,1 NAOMI K. COHN,2 DONALD J. NELSON,2 AND ROBERT S. KLEIN2

Departments of Medicine and Microbiology, Division of Infectious Diseases, University of Colorado Health Sciences Center, Denver, Colorado 802621; The Burroughs-Wellcome Company, Research Triangle Park, North Carolina 277092; and Laboratory of Organic Chemistry, Sloan-Kettering Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, New York, New York 100213

Received 8 September 1983/Accepted 18 November 1983

Purine metabolism in the pathogenic hemoflagellates is unique in that it can be inhibited by pyrazolopyrimidines and analogs. This is due, in part, to the fact that there is no de novo synthesis (2, 6, 10, 18, 31) and, more importantly, to the fact that the enzymes of the salvage pathways will accept the pyrazolopyrimidine ring as a purine. This property of the hemoflagellate enzymes does not occur in humans (7, 12, 22) and, for this reason, pyrazolopyrimidines offer promise as potential chemotherapeutic agents for the management of leishmaniasis and other forms of trypanosomiasis.

The prototype of this class of compounds is allopurinol (4-hydroxyxyprazolo[3,4-d]pyrimidine), which was the first such compound shown to be effective against leishmania (14, 21, 25) and subsequently against Trypanosoma cruzi (1, 16) and the African trypanosomes (15). Further investigation demonstrated that the ribonucleosides of pyrazolopyrimidines were as active as, and in some cases more active than, the corresponding bases. Allopurinol ribonucleoside (4-hydroxyxyprazolo[3,4-d]pyrimidine ribonucleoside) (24) and 4-thiopyrazolo pyrimidine ribonucleoside (17) were the first pyrazolopyrimidine ribonucleosides demonstrated to have antileishmanial activity in vitro. Subsequently, formycin B (7-hydroxyxyprazolo[4,3-d]pyrimidine ribonucleoside) was shown to have antileishmanial activity (5). Its metabolism is identical to that of allopurinol ribonucleoside (23, 26).

Since these compounds are inosine analogs (Fig. 1), we investigated several modifications of the inosine structure to determine which features of the molecule are important for activity against leishmania and trypanosomes yet prevent toxicity to mammalian hosts. The five-membered heterocyclic ring was chosen for modification since both the 3,4-d (allopurinol and thiopurinol ribonucleoside) and the 4,3-d (formycin B) configuration of the pyrazolopyrimidine ring were active against the hemoflagellates (Fig. 1).

Organisms were grown as described previously (8, 16, 18) in the presence or absence of the compounds in question (10 μg/ml), and the results were expressed as a percentage of the control. Doses effective against 50% of the organisms were determined by triplicate counting of organisms in a Coulter ZBI counter and determining a mean value. Agents which inhibited growth to less than 50% of the control were tested further by titration from 10 to 0.1 μg/ml. Concentration curves were done with mouse L cells as the mammalian model and with three pathogenic hemoflagellates: Leishmania donovani, Trypanosoma cruzi, and Trypanosoma gambiense. Two of the six, 9-deazainosine and allopurinol ribonucleoside, had very little toxicity for mouse L cells and offer promise as potential chemotherapeutic agents.

Previous investigations have suggested that inosine analogs would be good models for the development of chemotherapeutic agents active against pathogenic hemoflagellates. We have systematically modified the five-membered heterocyclic ring of six inosine analogs and tested them for their antiprotozoal activities and toxicity to a mammalian cell line. All six analogs were very active against the three protozoan pathogens Leishmania donovani, Trypanosoma cruzi, and Trypanosoma gambiense. The two of the six, 9-deazainosine and allopurinol ribonucleoside, had very little toxicity for mouse L cells and offer promise as potential chemotherapeutic agents.

* Corresponding author.
nous injection of formycin B is about 1,000 mg/kg (30). In that system, formycin B was two to four times less toxic than formycin A, the adenosine analog. It is known, however, to inhibit several enzymes of nucleic acid metabolism (20, 28) and, in mouse L cells, it is incorporated into RNA (T. Spector, T. E. Jones, S. W. LaFon, D. J. Nelson, R. L. Berens, and J. J. Marr, Biochem. Pharmacol., in press). This latter study demonstrated that mouse L cells are capable of phosphorylating formycin B and converting this nucleotide to all of the corresponding adenine nucleotide analogs. The conversion of formycin B to the nucleotides of formycin A raises the possibility that formycin A could be generated from formycin B in humans. Indeed, Berman et al. (4) have demonstrated that formycin A nucleotides can be formed from formycin B in human macrophages, and the toxicity of a series of formycin analogs to *L. tropica* was paralleled by their toxicities to these macrophages (3). Glazer and Lloyd (9) have shown that formycin A can be

![Pyrazolopyrimidine analogs of inosine.](image)

**FIG. 1.** Pyrazolopyrimidine analogs of inosine.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mouse L Cells</th>
<th>Leishmania donovani</th>
<th>Trypanosoma cruzi</th>
<th>Trypanosoma gambiense</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-Deazainosine</td>
<td>20</td>
<td>&lt;2</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Allopurinol riboside</td>
<td>&gt;2000</td>
<td>7</td>
<td>2-10</td>
<td>20</td>
</tr>
<tr>
<td>8-Azainosine</td>
<td>2</td>
<td>35</td>
<td>4.5</td>
<td>9</td>
</tr>
<tr>
<td>7-Thia 7,9-Dideazainosine</td>
<td>15</td>
<td>&lt;2</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

*Numbers represent effective doses for 50% of cells (micromolar concentrations).
incorporated into the DNA of human colon carcinoma cells. Formycin A is a cytotoxic analog of adenosine (11, 32) and is one of the most effective analogs to replace adenine in cellular reactions (33). The triphosphate of this compound can substitute for ATP as a substrate for aminoacyl-tRNA synthetase (33). Robbins et al. (27) have shown that formycin A has a close steric resemblance to adenosine and predicted that it would resemble the purine nucleoside in its enzymatic reactions. Formycin B also has shown some toxicity to mouse macrophage tissue culture (J774) (5). The evidence of toxicity for mammalian tissue culture systems was supported in this study since the toxic concentration for mouse L cells, although much higher than the 50% effective dose for the protozoans, was also much lower than that found for allopurinol ribonucleoside and 9-deazainosine.

This latter nucleoside is of considerable interest not only because it supports the general theme of inosine analogs as potential chemotherapeutic agents for several protozoa diseases but also because it has a carbon-carbon bond between the heterocyclic ring and the ribose. This bond is not broken in mammalian cells (11, 33), and 9-deazainosine should resist metabolic degradation in humans. The toxicity of this compound is low in mouse L cells, in excess of 1 mM, and it compares favorably with allopurinol ribonucleoside in this regard. The latter has been shown to be without toxicity to animals and humans in relatively high doses (unpublished data).

These data and published investigations indicate that inosine analogs are very reasonable models for the design of agents with antiprotozoal activity. The metabolism by and toxicity to animal cells will vary from one compound to another, and, at this time, cannot be predicted from the structural formulas. If the inosine analogs are converted to the corresponding adenine analog by mammalian cells, these agents probably will be very toxic (29). It has been shown, for example, that 9-deazainosine is quite toxic to human pancreatic carcinoma and human colon carcinoma cells (M. Y. Chu, L. B. Landry, and G. W. Crabtree, Abstr. Proc. Am. Assoc. Cancer Res., p. 306, 1983; R. I. Glazer and K. Hartman, Abstr. Proc. Am. Assoc. Cancer Res., p. 294, 1983), owing presumably to the fact that it does not react well with mammalian adenine deaminases. In this study with human pancreatic carcinoma cells, it was shown that the toxicity was due to the incorporation of this compound into nucleic mono-, di-, and triphosphates, and RNA. It also inhibited the biosynthesis of nucleic acid phosphodervatives and DNA (Chu et al., Abstr. Proc. Am. Assoc. Cancer Res.). The investigation with human colon carcinoma cells also demonstrated that 9-deazainosine was toxic and that this toxicity was associated with the incorporation of the compound into RNA and DNA (Glazer and Hartman, Abstr. Proc. Am. Assoc. Cancer Res.). An inosine analog which can be phosphorylated and aminated by a protozoan system, but not by a mammalian system, would be an excellent candidate agent for antiprotozoan chemotherapy.

This investigation received financial support from grants T16/181/78/30 and T16/181/3/29 from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases, Public Health Service grants AI-15663091 and AI-17970-01 from the National Institutes of Health, and the Burroughs-Wellcome Co.

LITERATURE CITED


