Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Suppl. I Vol. 83, November, 1988/Page 301

HYPOXANTHINE AND INOSINE ANALOGUES AS CHEMOTHERAPEUTIC AGENTS IN CHAGAS' DISEASE

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Caxambu, Brazil
November 7-9,1988

Introduction

The ability of pyrazolopyrimidines to inhibit the growth of members of the family Trypanosomatidae has raised exciting questions with respect to both basic biochemistry anhd chemotherapy. Mamalian species synthesize purines de novo and, in addition, have the capacity to salvage purine bases and nucleosides for nucleic acid and coenzyme synthesis. Leishmania and Trypanosoma species lack the ability to synthesize purines de novo but make use of active salvage machanisms for purine incorporation (1-5). These important biochemical differences between mammals and these protozoans have led to a more comprehensive understanding of the biochemistry of the organisms and may lead to some practical applications in the form of chemotherapeutic agents for Chagas' disease.

Chemistry

Compounds which have shown the most promise are analogues of purines or purine nucleosides. Of these, two pyrazolopyrimidines and a C-nucleoside have been most attractive. Of the former group, allopurinol, an analogue of hypoxanthine, and allopurinol ribonucleoside, an analogue of inosine have demonstrated the greatest promise. Of the latter, 9-deazainosine has the greatest activity and the widest spectrum.

Pyrazolopyrimidines are structural analogues of purines in which there is an inversion of the nitrogen from the seven position of the purine ring to what would correspond to position eight. This alteration makes the compound a pyrazolo(3,4-d)pyrimidine and ssubstantially alters its metabolic fate. Despite their structural similarities to purines, allopurinol, allopurinol ribonucleoside, and their metabolic products are not incorporated into the nucleic acids of mammalian tissues.

The C-nucleoside 9-deazainosine differs from inosine by the substitution of a carbon for a nitrogen in the 9 position. This creates a carbon-carbon bond between the purine ring and the ribose whihc is very stable biochemically. It is not broken by mammalian or microorganism cells which have been studied.

Pyrazolopyrimidines

Comparison of epimastigotes, trypomastigotes, and amastigotes

All three forms of the parasite incorporate allopurinol into analogues of IMP, AMP, ADP, and ATP. Mammalian cells will incorporate it into the IMP analogue only. This suggests that it is

the aminated nucleotide which is toxic to the microorganism and not the inosinic acid analogue. This conclusion is corroborated by studies in Leishmania donovani in which toxicity of purine analogues to the parasite and mammalian cells was correlated directly with the ability of the cell to aminate the compound into an analogue of adenine nucleotides (6).

Effects on nucleic acid metabolism,protein synthesis, and polysome formation.

Allopurinol inhibits the incorporation of [^{3}H] uracil and [^{14}C] leucine into RNA and protein. Aminopyrazolopyrimidine, an analogue of adenine which is converted into the nucleotide by T. cruzi, inhibits both RNA and protein synthesis completely at a concentration of 10 µgm/ml. At this concentration allopurinol inhibits both by approximately 40%. T. cruzi treated for 24 hrs. with allopurinol (25 µgm/ml) has a decrease in the maximum number of ribosomes per strand of RNA from 12 to 7. This suggests a premature termination of protein synthesis (RL Berens, JJ Marr, D.Lanar, and FS Cruz-unpublished data).

9-deazainosine

This C-nucleoside is very active against many protozoan pathogens. Its mechanism of action is analagous to that of the pyrazolopyrimidines mentioned above. It is phosphorylated to 9-deazaIMP and then aminated to the adenine nucleotide analogues. This metabolic transformation is carried out in leishmania, both promastigotes and amastigotes in U937 tissue culture (7,8), in T. gambiense procyclic forms (9), and in T. cruzi epimastigotes and amastigotes growing in VA-13 cells (7,10). It has been tested in monkeys and shown to be very effective against visceral leishmaniasis (11). This broad spectrum of activity has been extended to include the organism Pneumocystis carinii, a common cause of pneumonia in persons with AIDS. Experiments in vitro have shown that this compound can inhibit the growth of these organisms in WI-38 cells (12); moreover, infected rats treated with 9-deazainosine do not develop the pneumonia which occurs in the untreated controls. Histologic examination of the lungs of these animals shows that the organisms are dramatically reduced in number or are absent (13). The agent is also effective when given to rats prophylactically.

In T. cruzi, 9-deazainosine at a concentration of 5 µgm/ml will eliminate organisms from a tissue culture system. After a fourteen day exposure to the compound the cells are free of amastigotes and remain so during one additional month of observation after the drug was withdrawn. The metabolism of this compound in T. cruzi in

tissue culture is as described above. Further investigations on this interesting compound are in progress with T. cruzi.

The evidence to date indicates that 9-deazainosine is an agent with a broad activity against the hemoflagellates and P. carinii. The compound is difficult to synthesize chemically and this is a disadvantage. At this time two investigative groups are attempting to simplify the synthesis in order to provide enough material to use in animal studies.

Biological Activity of Pyrazolopyrimidines

How does the metabolism of pyrazolopyrimidines and the appearance of products which inhibit various enzymes correlate with biological activity? When studied in a tissue culture model, allpurinol, at a concentratin of 25 µgm/ml was able to cure the cell culture infected with T. cruzi (14). In a later investigation it was shown that there may be a natural resistance of this organism to the inosine analogues allopurinol riboside, formycin B, and 9-deazainosine but not to allopurinol, an analogue of hypoxanthine (15).

In an experimental study of allopurinol in mice with an acute infection with T. cruzi, Avila and Avila (16) showed that allopurinol, administered in ten daily doses of 32-64 mg/kg/day intraperitoneally protected mice from the acute form of this disease. By direct examination, parasitemia was undetectable for 310 days; subinoculation showed that there were small numbers of circulating trypanosomes. Mice which received a second schedule of treatment were cured of infection when tested as long as 275 days after treatment.

Despite the evidence in vitro, in tissue culture, and in mice that allopurinol is an effective agent against T. cruzi, there are other data by Pires et al (17) who used allopurinol in the treatment of dogs and compared the results with those obtained using nifurtimox. These investigators showed that the animals did not respond to either allopurinol or nifurtimox at four different experimental regimens. Another study by Berens et al (15) showed that DBA/2 mice treated with allopurinol riboside at 2 mg/ml in the drinking water had no signs of clinical illness nor evidence of parasitemia. They did, however, remain positive on subculture.

Thus it appears that pyrazolopyrimidine analogues of both hypoxanthine and inosine are effective in eliminating all signs and

symptoms of the disease in the animals studied, with the exception of that of Pires et al where neither allopurinol nor nifurtimox were active. This suggests that subclinical parasitemia may remain although no clinical disease is evident.

Studies in Humans

One study has been done in humans with chronic Chagas' Disease. An investigation by Gallerano et al (18) demonstrated that allopurinol at doses of 300 or 600 mg/day for 60 days could eliminate parasitemia as measured by xenodiagnosis. Since there were no clinical symptoms in the patients it was not possible to demonstrate clinical improvement. There were no serological data presented in a manner to show reversion to negativity or a decrease in titer during the course of treatment.

Subsequently, these patients were restudied in a retrospective manner to determine serologic findings in serum taken pretreatment and frozen. They also have been followed for two more years with repeated xenodiagnosis and serology. Preliminary analysis of these data has demonstrated a significant difference between untreated patients and those treated with allopurinol, 600 mg/day, or nifurtimox, or benznidazole. Similarly, there was a difference in the reversion to negativity in the complement fixation, immunofluroescence, and the hemagglutination test compared to the untreated group (Sosa, Gallerano, and Marr, unpublished data). This investigation is continuing with a new group of patients using allopurinol at a dose of 900 mg/day. The new investigation incorporates tests to follow parasitemia as well as the above serological tests and the lytic antibody response.

Summary

Analogues of purines, especially those of hypoxanthine and inosine, appear to hold considerable promise for the treatment of Chagas' Disease.

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