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# Pyrazolopyrimidine Metabolism in Leishmania and Trypanosomes: Significant Differences Between Host and Parasite

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The pathogenic hemoflagellates of the genera Leishmania and Trypanosoma are major causes of human disease in the tropical and subtropical areas of the world. In general, the agents used to treat diseases caused by these organisms are toxic and not suitable for administration to the millions of people infected. Investigations over the past several years have shown that there are several major differences between man and these protozoans with respect to purine metabolism. The differences appear to offer promise for the development of effective chemotherapeutic compounds. These organisms do not synthesize purines de novo, as does man. They are able to concentrate pyrazolopyrimidines within the cell and metabolize them as purines through the salvage pathways, ultimately incorporating them into nucleic acids. This does not occur in mammals. The pyrazolopyrimidine base allopurinol, which has served as a prototype, is activated by a phosphoribosyltransferase to the ribonucleotide. The ribonucleotide is aminated to the 4-aminopyrazolopyrimidine ribonucleotide and subsequently phosphorylated to the triphosphate form and incorporated into RNA. The pyrazolopyrimidine ribonucleosides formycin B and allopurinol ribonucleoside are activated through a nucleoside phosphotransferase. The resulting ribonucleotide is aminated and incorporated into RNA as described above. These metabolic peculiarities occur not only in the forms of these parasites which are found in the insect vectors but also in the intracellular forms which are pathogenic in man. The differences in the enzymology and metabolism of purines which exist in the genera Leishmania and Trypanosoma offer excellent opportunities for chemotherapeutic exploitation.

**Key words: trypanosome, purine, pyrazolpyrimidine, metabolism, leishmania, chemotherapy**

Abbreviations used: HPP, 4-hydroxypyrazolo (3,4-d) pyrimidine (allupurinol); APP, 4-aminopyrazolo (3,4-d) pyrimidine (aminopurinol); HPPR, 4-hydroxypyrazolo (3,4-d) pyrimidine ribonucleoside (allopurinol riboside); HPPR-MP, 4-hydroxypyrazolo (3,4-d) pyrimidine ribonucleoside monophosphate; APPR-MP, 4-aminopyrazolo (3,4-d) pyrimidine ribonucleoside monophosphate; APPR-DP, 4 aminopyrazolo (3,4-d) pyrimidine ribonucleoside diphosphate; APPR-TP, 4-aminopyrazolo (3,4-d) pyrimidine ribonucleoside triphosphate; TPP, 4-thiopyrazolo (3,4-d) pyrimidine (thiopurinol); TPPR, 4-thiopyrazolo (3,4-d) pyrimidine ribonucleoside (thiopurinol riboside); TPPR-MP, 4-thiopyrazolo (3,4-d) pyrimidine ribonucleoside monophosphate; FORB, 7-hydroxypyrazolo (4,3-d) pyrimidine ribonucleoside; FORA, 7-aminopyrazolo (4,3-d) pyrimidine ribonucleoside; FORB-MP, 7-hydroxypyrazolo (4,3-d) pyrimidine ribonucleoside monophosphate; FORA-MP, 7-aminopyrazolo (4,3-d) pyrimidine ribonucleoside monophosphate.

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The hemoflagellates that infect man have proven recalcitrant to effective chemotherapy since the specificity found in agents used in the treatment of bacterial diseases is lacking in protozoan diseases. A high therapeutic index for a given antimicrobial usually requires a specific, although not necessarily major, difference in the metabolism between the host and the parasite. Such a difference may exist in the ability of leishmania and trypanosomes to metabolize the pyrazolopyrimidine nucleus as though it were a purine base. The ability of pyrazolopyrimidines to inhibit the growth of members of Trypanosomatidae has raised some exciting questions of basic biochemistry and chemotherapy. Mammalian cells synthesize purines de novo and have the capacity to salvage purine bases and nucleosides for nucleic acid and coenzyme synthesis. Leishmania spp and Trypanosoma spp cannot synthesize purines de novo and use salvage mechanisms for purine incorporation [1–5]. These differences and those in the specificities of enzymes in the purine salvage pathways, with respect to pyrazolopyrimidines, have led to a more comprehensive understanding of the biochemistry of these organisms.

## CHEMISTRY

Pyrazolopyrimidines are structural analogs of purines in which there is an inversion of the nitrogen from the 7 position of the purine ring to what would correspond to position 8. This makes the compound a pyrazolo (3,4-d)pyrimidine and substantially alters its metabolic fate (Fig. 1). Allopurinol (4-hydroxypyrazolo [3,4-d]pyrimidine; HPP) is structural analog of hypoxanthine. It is a substrate for and an inhibitor of xanthine oxidase and is converted by that enzyme to oxipurinol (4,6-dihydroxypyrazolo [3,4-d]pyrimidine), a more effective inhibitor.

The metabolism of HPP in man differs from that in the hemoflagellates. About 60% is rapidly converted to oxipurinol. Thirty percent, in the steady state, will be excreted in the urine as allopurinol and the remaining 10% as allopurinol-1-ribonucleoside (HPPR) [6]. Besides HPPR, two other ribonucleosides, 1-ribosyloxipurinol and 7-ribosyloxipurinol, are formed as minor metabolic products by the action of phosphatases on ribonucleotides or the action of nucleoside phosphorylases [7,8]. The ribonucleotides formed from these are potential inhibitors of orotidylate decarboxylase [9] but there is little or no effect on pyrimidine metabolism in man or animals [10]. The possible effects of allopurinol on purine metabolism have been subject to considerable investigation but none have been noted [11–13]. There is no evidence for incorporation of any allopurinol derivative into nucleic acid [14].

## METABOLISM OF PYRAZOLOPYRIMIDINES IN LEISHMANIA

Allopurinol inhibition of the growth of leishmania was first demonstrated by Pfaller and Marr in L braziliensis [15], and subsequently in L donovani and L mexicana [16]. The investigation developed from an observation by Frank et al [17] that allopurinol could inhibit the growth of Crithidia fasciculata. The antileishmanial effect was reversed in all three leishmania by adenine, or its metabolic precursors or derivatives, and it was proposed that the adenylosuccinate synthetase or the adenine phosphoribosyltransferase (APRTase) might be the site of action for these agents [16].

Subsequent investigations documented the metabolic conversions of allopurinol in these organisms (Fig. 2). The promastigotes of L donovani and L braziliensis

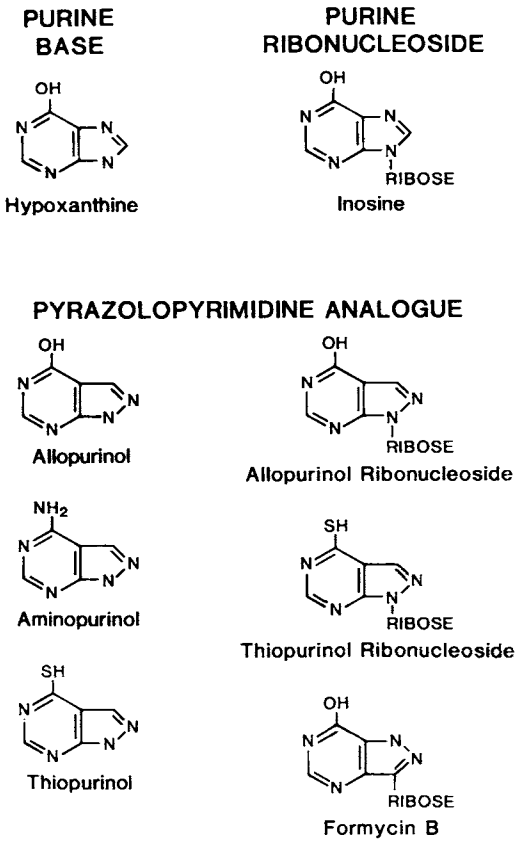


Fig. 1. Structures of certain purines and pyrazolopyrimidines. [Reproduced with permission from Marr JJ, Berens RL: Mol Biochem Parasitol 7:339, 1983.]

were capable of concentrating HPP from the medium 40- to 100-fold and formed millimolar concentrations of allopurinol ribonucleoside-5′-monophosphate (HPPR-MP) [18]. The intracellular concentration of HPPR-MP was 2–3 mM, about 10× that of allopurinol, approximately the same as adenosine triphosphate [ATP], indicating that the purine economy of the cell had been altered greatly. HPPR-MP was aminated to 4-aminopyrazlopyrimidine-5′-monophosphate (APPR-MP), converted to the di- and triphosphates, APPR-DP and APPR-TP, respectively, and the latter was incorporated into the cellular RNA [18] (Fig. 2). This incorporation of APPR-TP into RNA of the parasite is unique. Mammalian cells show neither this conversion nor the incorporation [8].

As noted above, adenine and hypoxanthine reversed the antileishmanial action of HPP [16]. It was subsequently shown that adenine aminohydrolase (EC 3.5.4.2; adenase) rapidly converted adenine to hypoxanthine and the latter was converted to

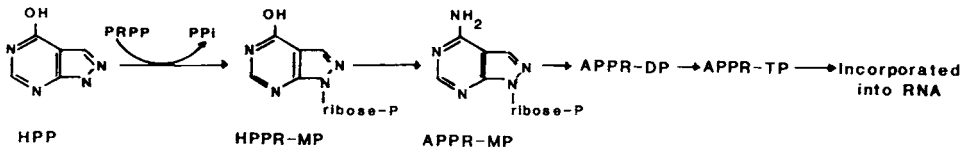


Fig. 2. Metabolic transformations of allopurinol. [Reproduced with permission from Marr JJ, Berens RL: Mol Biochem Parasitol 7:339, 1983.]

adenine and guanine nucleotides [1]. Adenine did not alter the transport of HPP into the organisms but did prevent its conversion to the ribonucleoside and ribonucleotide by competing with hypoxanthine for hypoxanthine-guanine PRTase (HGPRTase) [19]. There was no evidence of depletion of nucleotide pools in cells exposed to pyrazolopyrimidines.

The important feature which distinguishes the metabolism of pyrazolopyrimidines in these parasitic protozoans from that in man is the accumulation of large quantities of HPPR-MP. Mammalian cells accumulate only small quantities of this compound and none of the aminopyrazolopyrimidine compounds [8]. This accumulation is due to the substrate specificities of the PRTases in these organisms. For the hypoxanthine-guanine PRTase (HGPRTase) the apparent $K'_m$ values for guanine and hypoxanthine were similar but guanine was the preferred substrate. HPP was considerably less efficient as a substrate and 4-thiopyrazolopyrimidine (TPP), the thio-analogue of HPP, was even less so. The pyrazolopyrimidines, however, were more efficient substrates for the HGPRTase from this organism than for the enzyme from human erythrocytes by a factor of ten. This is consistent with the observation that L. donovani promastigotes accumulate large quantities of HPPR-MP and human cells do not [19]. This probably is not sufficient to account for the approximately 100-fold concentration of HPPR-MP in the parasite. Part of this may be accounted for by the two- to 70-fold larger amount of HGPRTase in L donovani promastigotes compared to mammalian cells [8–11] and by the absence of xanthine oxidase. In addition, these calls have the capacity to accumulate HPP from the medium to achieve intracellular concentrations of approximately 0.1–0.3 mM. This could change the $S/K'_m$ ratio within the cell to make HPP a competitive substrate.

Approximately 10% of HPPR-MP is converted to APPR-MP in L donovani. This indicates that the succino-AMP synthetase (EC 6.3.4.4) and the succino-ampy-lase AMP lyase (EC 4.3.2.2) are capable of aminating HPPR-MP in this organism. Investigation of these enzymes [20] showed that the synthetase would accept HPPR-MP as a substrate. This compound is not a substrate of succino-AMP synthetase from a mammalian source [21]. The synthetase appears to be the rate-limiting enzyme. The protozoan enzymes may be unique in their abilities to accept HPPR-MP as an alternate substrate for inosinic acid (IMP). There is no evidence for this transformation in mammalian tissues treated with HPP [8,22]. The succino-AMP lyase from this organism, however, is similar to its counterparts from a variety of sources. The condensed products formed from IMP or HPPR-MP are cleaved efficiently.

The amination of HPPR-MP, despite the unfavorable kinetic constants, is due to the high concentration of this compound within the cell, about 100-fold higher than that of IMP. The intracellular $S/K'$ ratio of five to ten for this compound is higher

than that for IMP (approximately three) and allows HPPR-MP to compete favorably for catalysis. The low $V_{max}$ for HPPR-MP (about 1% that for IMP) is consistent with the metabolic studies which showed that APPR-MP is formed very slowly after the rapid accumulation of HPPR-MP. A similar situation exists in T cruzi.

The guanosine monophosphate (GMP) reductase (EC 1.6.6.8) is inhibited by both HPPR-MP and TPPR-MP [23]. Two other compounds were competitive inhibitors of the GMP reductase from L donovani, xanthosine monophosphate (XMP), and IMP. The K′ for these compounds were 14, 14, 37, and 34 $\mu$M, respectively. This is a major difference between the GMP reductase of the parasite and that derived from a human source [24]. That from human erythrocytes is unresponsive to pyrazolopyrimidines; the K for HPPR-MP is 3,600 $\mu$M and that for TPPR-MP is 680 $\mu$M. The kinetic data suggest that inhibition of this enzyme by pyrazolopyrimidine ribonucleotides should be virtually complete in the parasite since the concentration of these compounds in the cell is 1–2mM. Such inhibition may create an imbalance in the ratio of adenine to guanine nucleotides.

## ALLOPURINOL RIBONUCLEOSIDE

In man, about 10% of HPP is converted to allopurinol ribonucleoside [22]. HPPR is not oxidized by xanthine oxidase but is oxidized by aldehyde oxidase, which is present only in low levels in humans [25,26]. For these reasons HPPR was studied as an antileishmanial agent. It was unexpectedly active and its metabolism was studied in detail [27]. It is approximately tenfold more active against L braziliensis and 300-fold more active against L donovani than the parent compound. HPPR-MP was formed in large amounts from HPPR and the aminopyrazolopyrimidine ribonucleotides were made and incorporated into RNA. Very little HPPR was converted to any other metabolic product. After a four-day incubation of HPPR with L donovani, 92% of the ribonucleoside in the incubation medium was unchanged. Similar experiments showed that inosine was converted almost completely to hypoxanthine within 1 hr [1]. The direct phosphorylation of HPPR was due to a nucleoside phosphotransferase, rather than a nucleoside kinase, and the product was 5′-ribonucleotide. The metabolic transformations of HPP, HPPR, and APP are summarized in Figure 3.

## OTHER PURINE ANALOGUES

These results prompted an investigation of a large number of other substituted pyrazolopyrimidines and related compounds. Of these, only thiopurinol and its ribonucleoside showed promise [28]. TPP and HPP are of approximately equivalent antileishmanial activities. The ribonucleosides are also of comparable activities and are more active than the bases, except in L mexicana, where HPP and HPPR are of equal potency [27].

To study the metabolism of these compunds, the promastigotes of L donovani were incubated in the presence of [4-$^{35}$S] TPP, [4-$^{35}$S] TPPR, and [U-$^{14}$C] ribose-TPPR. The organisms did not metabolize TPP beyond the 5′-monophosphate stage (TPPR-MP) and there was no detectable conversion to APPR-MP nor to HPPR-MP. The thiol group was stable for the duration of the experiment (24 hr) and no radiolabeled sulfate was found. When TPPR was used as a precursor, radiolabeled

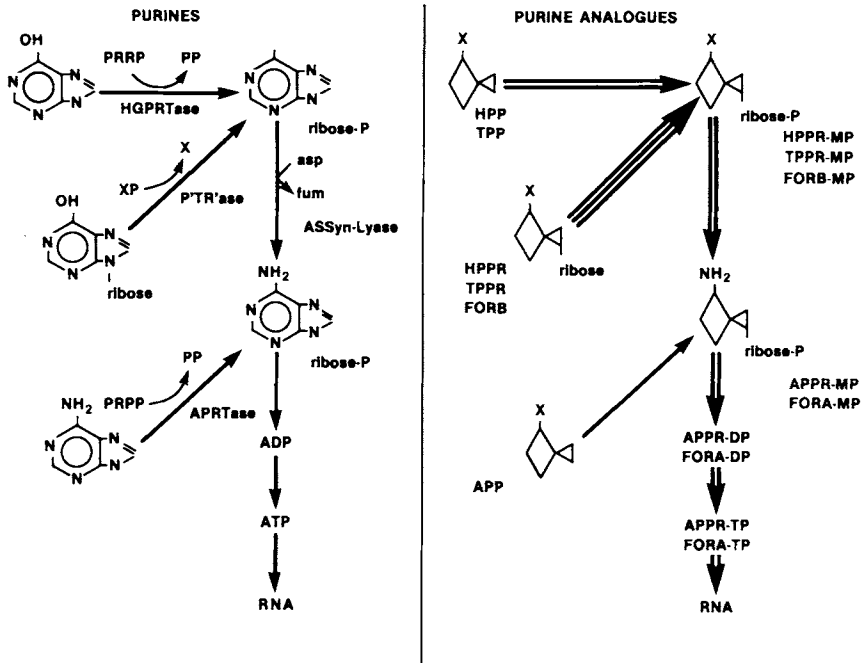


Fig. 3. Comparison of purine and pyrazolopyrimidine metabolism in hemoflagellates. The major salvage pathways for purines are depicted on the left and the corresponding transformations of the pyrazolopyrimidine analogues on the right. The abbreviatioons are as follows: HGPRTase, hypoxanthine-guanine-phosphoribosyltransferase; P'T'ase, nucleoside phosphotransferase; ASSYN/lyase, succinyl-AMP synthetase/lyase. Abbreviations for the pyrazolopyrimidine analogs are given in the text. All of the analogs are metabolized to the corresponding aminopyrazolopyrimidine triphosphate except for TPP and TPPR, which are halted at the thiopyrazolopyrimidine ribonucleotide. [Reproduced with permission from Marr, JJ, Berens RL: Mol Biochem Parasitol 7:339, 1983.]

either in the sulfur or ribose moiety, only radiolabeled TPPR-MP was found. The ribose remained with the pyrazolopyrimidine.

Since both TPP and TPPR were converted only to TPPR-MP, investigations were undertaken to clarify the metabolism. An earlier investigation demonstrated that TPP was a substrate for the HGPRTase of L dononvani [19]. Studies with the succino-AMP synthetase showed that the possible product, succino-APPR-MP was not formed [28]. The inability of TPPR-MP to serve as a substrate corroborated the studies in vivo which did not detect the formation of APPR-MP. Although TPPR-MP was unable to substitute for IMP in the reaction, it did inhibit the conversion of IMP. TPPR was phosphorylated by a nucleoside phosphotransferase and the kinetic data were similar to those found previously for HPPR [27,28].

The related inosine analogue, formycin B (7-hydroxy-3-B-D-ribofuranosylpyrazolo[4,3-d]pyrimidine; FORB), also has antileishmanial activity. Formycin compounds are pyrazolopyrimidines with an inversion of the carbon and nitrogen atoms in positions 8 and 9 of the five-membered ring (Fig. 1). This C-nucleoside analogue of inosine is neither phosphorylated nor cleaved in mammalian cells [29,30]. A study of the effects of FORB on leishmania was published by Carson and Chang [31]. They showed that FORB, at a concentration of 1 $\mu$M, inhibited the growth of L donovani

and L mexicana promastigotes. The promastigotes produced formycin B monophosphate (FORB-MP) but the AMP analogue, formycin A monophosphate (FORA-MP), was not found.

A subsequent study by Nelson et al [32] confirmed that FORB was an effective antileishmanial agent but also demonstrated that these organisms converted FORB to the ribonucleotides of FORA, indicating that the metabolic pathway is exactly the same as that for HPPR. The conversion of FORB to FORA ribonucleotides was confirmed by Rainey and Santi [33]. The metabolism of FORB and HPPR in leishmania appear to be identical.

## EFFECT OF PYRAZALOPYRIMIDINES ON AMASTIGOTES OF L DONOVANI

Since the intracellular parasite, or amastigote, is the pathogenic form in man it is imperative to demonstrate that pyrazolopyrimidines have similar effects on it. Experiments to compare HPP metabolism included exposure of free amastigotes; minced, infected, hamster spleen preparations; and infected P388$D_1$ macrophage cultures with radiolabeled HPP. The amastigotes of L donovani, the infected hamster spleen, and infected macrophages produced HPPR-MP as well as the three ribonucleotides of APP. Under identical conditions uninfected spleen and macrophages yielded only radiolabeled HPPR-MP; no APP ribonucleotides were formed. When HPP or HPPR were added to infected P388$D_1$ cultures, both compounds eliminated the intracellular parasites [34]. When exposed to either TPP or TPPR, the percentage of infected macrophages decreased from 60% to approximately 15% within 7 days. At 15 days the infected cells exposed to both compounds still had an apparent infection rate of 15%; untreated cells were now 80% infected. When the infected, treated macrophages were placed at 26°C on day 15, in an attempt to recover intracellular oganisms, none grew. Corresponding experiments with untreated, infected macrophages yielded amastigotes which transformed to promastigotes [28]. None of these agents inhibited the growth of uninfected macrophages.

FORB at a concentration of 1 $\mu$M inhibited the growth of amstigotes of L mexicana in J774G8 macrophages by approximately 50%. When the concentration was increased to 10 $\mu$M the inhibition of growth of the macrophages was approximately 30%. FORB markedly decreased the number of amastigotes in the livers of hamsters infected with L donovani when the drug was given for 5 days [31].

## THE METABOLISM OF PYRAZOLOPYRIMIDINES IN TRYPANOSOMA CRUZI

### Similarities to Leishmania

The growth of the culture (epimastigote) form of Trypanosoma cruzi is inhibited by HPP [35]. Attempts to reverse its effects showed that only adenine, hypoxanthine, and inosine were effective. Subsequent studies showed that epimastigotes of the Costa Rica, CL, Peru, and Y strains ([35], Berens and Marr, unpublished results) as well as the Ma and $F_1$ [36] strains of T cruzi had the ability to convert HPP to APP ribonucleotides and incorporate the latter into RNA. After incubation with HPP, the intracellular concentrations of HPPR-MP were similar to those found with leishmania. There was no evidence of depletion of the nucleotide pools.

The enzymes responsible for these conversions have not been studied in as much detail as have those of L donovani. Gutteridge et al [37] reported that T cruzi

contains the same PRTases that were described for L donovani. Presumably the HGPRTase is responsible for the conversion of HPP to HPPR-MP while the APRTase converts APP to APPR-MP. Spector et al [38] described the kinetic properties of succino-AMP synthetase and lyase from T cruzi. The substrate specificity and the $K'_m$ of succino-AMP synthetase were essentially identical to those of L donovani but were clearly distinguishable from the mammalian enzyme. The succino-AMP lyase from T cruzi has the the same broad substrate specificity that seems to be characteristic of this enzyme from most organisms [20].

### Differences From Leishmania

While the effects and metabolism of HPP in T cruzi are similar to those found for leishmania, there are some differences between these organisms with respect to other pyrazolopyrimidines. HPPR has little effect on the growth of the Costa Rica (CR) strain of T cruzi and only small amounts of the compound are converted to HPPR-MP and APP ribonucleotides [35]. More recent investigations (Berens, Marr, Nelson, LaFon, unpublished results) have shown that the CL strain also does not respond to HPPR. There are strain differences, however, since the Peru and Y strains of T cruzi are sensitive to HPPR. At this writing it appears that some strains of T cruzi will respond to HPPR and others will not; the biochemical data suggest that this is related to the relative inabilities of some strains to metabolize the ribonucleoside.

In general, T cruzi shows the same response to FORB as it does to HPPR. Those strains which are sensitive to the latter also respond to the former; those resistant to one are resistant to both. As was found for L donovani, sensitive strains of T cruzi convert FORB to FORB-MP. This is followed by amination to FORA-MP and the di- and triphosphates. The enzymes responsible for this are presumed to be the same as those which interconvert pyrazolopyrimidines in leishmania and are currently under investigation.

### Comparison of Epimastigotes, Trypomastigotes, and Amastigotes

Since the pathogenic forms of T cruzi are the amastigote and trypomastigote it is important to know whether pyrazolopyrimidine metabolism in these is the same as in the epimastigote. Bloodstream trypomastigotes (Peru strain) isolated from infected chinchillas, amastigote-infected spleen cells from infected chinchillas, and transformed human diploid lung cells (VA-13) infected with T cruzi all produce significant amounts of HPPR-MP, APPR-MP, APPR-DP, and APPR-TP. Thus, both the amastigotes and trypanomastigotes possess the same metabolic sequence as described for epimastigotes and the promastigotes and amastigotes of leishmania. Treatment of infected VA-13 cultures with HPP (25 $\mu$g/ml) eradicated the infection [39]. Three subcultures from the original infection in the presence of the drug were required. This agrees with the findings of Avila and Avila that T cruzi-infected mice treated with allopurinol showed a very significant increase in survival time compared to infected controls [40,41].

## CHEMOTHERAPEUTIC POTENTIAL

Metabolism of pyrazolopyrimidines by enzymes of the purine salvage pathways is a characteristic common to all of the hemoflagellates studied thus far. This

metabolic peculiarity may offer opportunities for chemotherapy of diseases cause by some of them. Although the biochemistry of purine and pyrazolopyrimidine metabolism is relatively well understood, the potential chemotherapeutic benefits remain to be demonstrated. The in vitro studies have been encouraging. To date, there have been relatively few studies in animals. A study in mice using L major and L mexicana amazonensis demonstrated that allopurinol was an effective agent in this model [42]. A study in the Aotus monkey demonstrated that allopurinol, given orally, was an effective agent against L braziliensis panamensis [43]. A small clinical study documented some efficacy of allopurinol in humans with antimony-resistant, visceral leishmaniasis [44]. However, these patients have not had prolonged follow-up and more time must pass before this study can be evaluated critically. One animal study by Avila and Avila [41] demonstrated that allopurinol was effective in treating acute Chagas's disease in mice. All of these studies were done with allopurinol and initiated before it was recognized that allopurinol riboside was much more active in vitro. At this writing a clinical trial of the ribonucleoside is being organized which will clarify the role that it may have in the management of leishmaniasis.

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## REFERENCES

1. Marr JJ, Berens RL, Nelson DJ: Biochim Biophys Acta 44:360, 1978.
2. Trager W: Ciba Found Symp 20:225, 1974.
3. Gutteridge WE, Gaborek N: Int J Biochem 10:415, 1979.
4. Ceron CR, Caldas RA, Feliz CF, Mundim MH, Roitman I: J Protozool 26:479, 1979.
5. Berens RL, Marr JJ, LaFon SW, Nelson DJ: Mol Biochem Parasitol 3:187, 1981.
6. Elion GB, Kovensky A, Hitchings GH: Biochem Pharmacol 15:863, 1966.
7. Krenitsky TA, Strelitz RA, Hitchings GH: J Biol Chem 242:2675, 1967.
8. Nelson DJ, Bugge CJL, Krasny HC, Elion GB: Biochem Pharmacol 22:2003, 1973.
9. Beadmore TD, Kelley WN: J Lab Clin Med 78:696, 1971.
10. Fyfe JA, Nelson DJ, Hitchings GH: In Sperling O, DeVries A, Wyngaarden JB (eds): "Purine Metabolism in Man." New York: Plenum, 1974, pp 621–628.
11. Fox IH, Wyngaarden JB, Kelley WN: N Engl J Med 283:1177, 1970.
12. Krenitsky TA, Papaioannou R, Elion GB: J Biol Chem 244:1263, 1969.
13. Elion GB, Nelson DJ: In Sperling O, DeVries A, Wyngaarden JB (eds): "Purine Metabolism in Man." New York: Plenum, 1974, pp 639–652.
14. Hitchings EH: Arthritis Rheum 18:863, 1975.
15. Pfaller MA, Marr JJ: Antimicrob Agents Chemother 5:469, 1974.
16. Marr JJ, Berens RL: J Infect Dis 136:724 1977.
17. Frank O, Baker H, Hutner SH: J Protozool 17:153, 1970.
18. Nelson DJ, Bugge CJL, Elion GB, Berens RL, Marr JJ: J Biol Chem 254:3959, 1979.
19. Tuttle JV, Krenitsky TA: J Biol Chem 255:909, 1980.
20. Spector T, Jones TE, Elion GB: J Biol Chem 254:8422, 1979.
21. Spector T, Miller RL: Biochim Biophys Acta 455:509, 1976.
22. Elion GB: In Kelley WN, Weiner IM (eds): "Handbook of Experimental Pharmacology." New York: Springer-Verlag, 1978, Vol 51, pp 485–514.
23. Spector T, Jones TE: Biochem Pharmacol (in press), 1982.

24. Spector T, Jones TE, Miller RL: J Biol Chem 54:2308, 1980.
25. Krenitsky TA, Neil SM, Elion GB, Hitchings GH: Arch Biochem Biophys 150:585, 1972.
26. Krenitsky TA, Tuttle JV, Cattau EL, Wang P: Comp Biochem Physiol 49B:687, 1974.
27. Nelson DJ, LaFon SW, Tuttle JV, Miller WH, Miller RL, Krenitsky TA, Elion GB, Berens RL, Marr JJ: J Biol Chem 254:11544, 1979.
28. Marr JJ, Berens RL, Nelson DJ, Krenitsky TA, Spector T, LaFon SW, Elion GB: Biochem Pharmacol 31:143, 1982.
29. Umezawa H, Sawa T, Fukagawa Y, Homma I, Ishizuka M, Takeuchi T: J Antibiot (Tokyo) 20:308, 1967.
30. Sheen MR, Kim BK, Parks RE: Mol Pharmacol 4:293, 1968.
31. Carson BA, Chang K-P: Biochem Biophys Res Commun 100:1377, 1981.
32. Nelson DJ, LaFon SW, Jones TE, Spector T, Berens RL, Marr JJ: Biochim Biophys Res Commun 108(1):349, 1982.
33. Rainey P, Santi DV: Proc Natl Acad Sci USA 80:288, 1983.
34. Berens RL, Marr JJ, Nelson DJ, LaFon SW: Biochem Pharmacol 29:2397, 1980.
35. Marr JJ, Berens RL, Nelson DJ: Science 201:1018, 1978.
36. Avila JL, Avila A, Casanova MA: Biochem Parasitol 4:265, 1981.
37. Gutteridge WE, Davies MJ: FEMS Microbiol Lett 13:207, 1982.
38. Spector T, Berens RL, Marr JJ: Biochem Pharmacol 31:225, 1982.
39. Berens RL, Marr JJ, Cruz FS, Nelson DJ: Antimicrob Agents Chemother 22:657, 1982.
40. Avila JL, Avila A, Minoz E: Am J Trop Med Hyg 30:769, 1981.
41. Avila JL, Avila A: Exp Parasitol 51:204, 1980.
42. Peters W, Trotter ER, Robinson BL: Ann Trop Med Parasitol 74:321, 1980.
43. Walton BC, Harper J, Neal RA: Am J Trop Med Hyg 32:46, 1983.
44. Kager PA, Rees PH, Wellde BT, Hockemeyer WT, Lyerly WH: Trans R Soc Trop Med Hyg 75:556, 1981.