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# Activity of Purine Analogs Against *Leishmania tropica* Within Human Macrophages In Vitro

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The activity of purine analogs against *Leishmania tropica* in human monocyte-derived macrophages in vitro was determined. Formycin B, formycin A, formycin B and A monophosphate, and formycin A triphosphate all had 50% effective doses of 0.02 to 0.04 μM and eliminated 90% of organisms at ≤0.5 μM. Allopurinol ribonucleoside was much less active: the 50% effective dose was 76 to 190 μM, and 90% of organisms were not eliminated at the highest dose tested (190 μM). 7-Deazainosine had a low 50% effective dose (0.2 μM), but only 80% of organisms were eliminated at 4 μM. Thio derivatives were as active as or less active than the parent compounds. These data suggest that certain inosine analogs are much more active than others against macrophage-contained *Leishmania* spp. such as are found in human lesions. However, because toxicity to the human macrophage hosts generally paralleled antileishmanial activity, the more active compounds might also be more toxic to human cells. The activity of 3-deazaguanosine (50% effective dose, 3.6 μM) in this model suggests that guanosine derivatives may have potential as antileishmanial agents.

The need for orally administrable, nontoxic antileishmanial agents has led to investigation of the antileishmanial activity of hypoxanthine and inosine analogs such as allopurinol, allopurinol ribonucleoside, and formycin B. In vitro, these agents are at least partially active against promastigotes and against amastigotes within macrophages, a clinically comparable model (2–4, 6, 10, 13). In vivo, allopurinol is active against mucous leishmaniasis in *Aotus* monkeys (15), and formycin B is active against visceral disease in hamsters (6; J. D. Berman, C. Keenan, S. Lamb, W. Hanson, and V. Waits, Exp. Parasitol., in press). Allopurinol is presently in trial against human visceral disease (9). In vitro biochemical studies have shown that the antileishmanial activity of these agents is due to metabolism of the drugs into analogs of inosine and adenosine nucleotides by the organisms (2, 6, 12–14). Inhibition of guanosine nucleotide utilization may also be important for antileishmanial activity since mycophenolic acid, an inhibitor of guanosine monophosphate synthesis from inosine monophosphate, inhibits amastigote multiplication in vitro (4).

A systematic investigation of the antileishmanial activity of purine analogs has not been reported. In the present work we determined and compared the activity of inosine, adenosine, and guanosine analogs against *Leishmania* amastigotes within human macrophages in vitro. This model is one in which clinically employed agents are active (5) and in which hypoxanthine metabolism is comparable to that of cells in general (4).

## MATERIALS AND METHODS

**Exposure of infected macrophages to purine analogs.** Human macrophage cultures were derived from the monocytes of the peripheral blood of normal human volunteers by methods previously described (5). After being infected with amastigotes of *Leishmania tropica* WR 401 (NIH 173), infected macrophage cultures in 1.0 ml of culture medium were exposed to a constant dose of a purine analog for 6 days. The culture medium used was RPMI-1640 (GIBCO Laboratories, Grand Island, N.Y.) containing 10% heat-inactivated fetal calf serum (GIBCO Laboratories), penicillin (50 U/ml), and streptomycin (50 μg/ml). After 6 days, the number of amastigotes per 100 macrophages in control (non-drug-treated) cultures and experimental cultures was determined by counting 100 to 200 Giemsa-stained macrophages in each culture. The number of macrophages per culture was estimated by counting 20 representative fields for each culture. In initial experiments, drug doses of 0.01 to 1.0 μM were employed. Generally, the drug dosage was increased in subsequent experiments until macrophage toxicity (see below) or a dose of at least 70 μM was achieved.

**Enumeration of data.** The number of *Leishmania* amastigotes per 100 macrophages surviving in drug-treated cultures was expressed as a percentage of the

number in simultaneously cultivated controls. The concentration of drug calculated to eliminate 50% of amastigotes compared to controls (the 50% effective dose [$ED_{50}$]) was determined by nonlinear regression analysis (7) of the results of each experiment. For drugs for which the dose-response curve was so flat that statistical analysis could not be performed, the $ED_{50}$ was estimated by inspection of the data. The doses of drug that eliminated 90% or more of the organisms (>90% effective dose) and that resulted in cytolysis and loss of 50% or more of the macrophages from the culture surface (macrophage toxic dose) were determined by inspection of the data.

**Antileishmanial agents.** Formycin B, formycin A, formycin B monophosphate, formycin A monophosphate, and formycin A triphosphate were purchased from Calbiochem-Behring, La Jolla, Calif. All other drugs were obtained from the drug inventory of the Walter Reed Army Institute of Research, Washington, D.C. The drugs were dissolved in culture medium at 50 to 1,000 times the concentrations used for experimentation and were sterilized by filtration (0.45 μM filter, Millipore Corp., Bedford, Mass.). To ensure drug stability, stock solutions were stored at 5°C and used

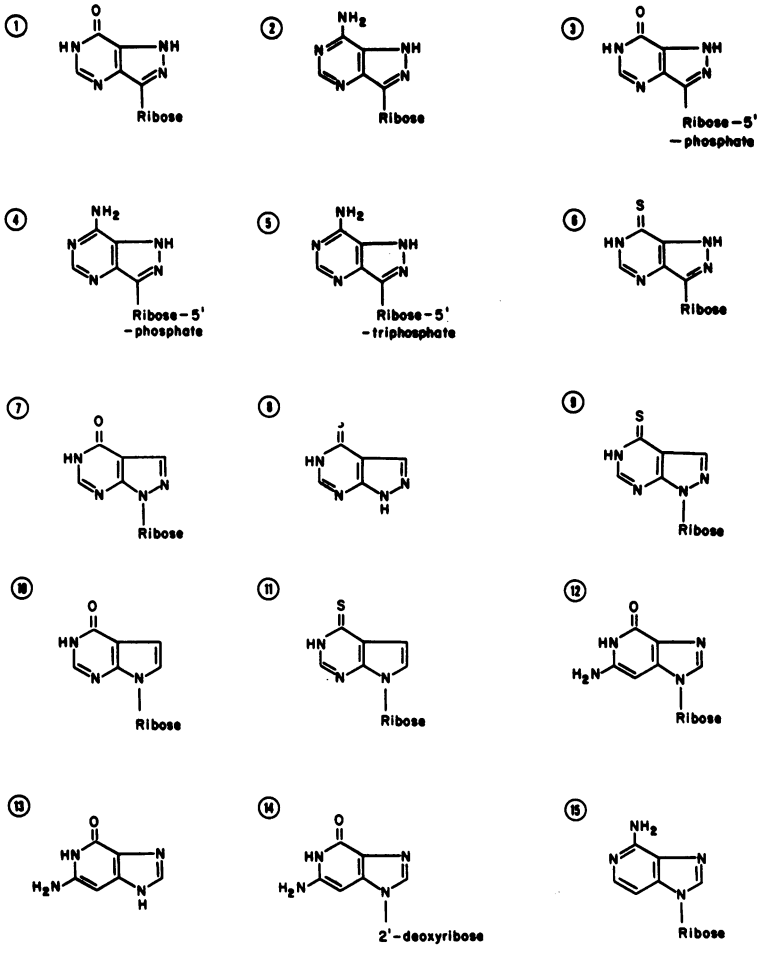


FIG. 1. Structures of compounds tested. The proper names for these compounds are as follows: 1, Formycin B(3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7-one); 2, formycin A(7-amino-3-β-D-ribofuranosylpyrazolo-[4,3-*d*]pyrimidine); 3, formycin B 5'-monophosphate(3-β-D-ribofuranosylpyrazolo[4,3-*d*]-pyrimidin-7-one 5'-monophosphate); 4, formycin A 5'-monophosphate(7-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine 5'-monophosphate); 5, formycin A 5'-triphosphate(7-amino-3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine 5'-triphosphate); 6, thioformycin B(3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidin-7-thione); 7, allopurinol ribonucleoside(1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-one); 8, thiopurinol(pyrazolo[3,4-*d*]pyrimidin-4-thione); 9, thiopurinol ribonucleoside(1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4-thione); 10, 7-deazainosine(7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidin-4-one); 11, thio-7-deazainosine(7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidin-4-thione); 12, 3-deazaguanosine(6-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridin-4(5H)-one); 13, 3-deazaguanine(6-aminoimidazo[4,5-*c*]pyridin-4-(5H)-one); 14, 3-deaza-2'-deoxyguanosine(6-amino-1-(1-deoxy-β-D-*erythro*-pentofuranosyl)imidazo[4,5-*c*]pyridine-4(5H)-one); 15, 3-deazaadenosine(4-amino-1-β-D-ribofuranosylimidazo[4,5-*c*]pyridine).

for only 2 weeks (two experiments). Structures of the drugs tested are shown in Fig. 1.

## RESULTS

The $ED_{50}$s for the elimination of *Leishmania* amastigotes from infected macrophages exposed to formycin B, formycin A, formycin B monophosphate, formycin A monophosphate, or formycin A triphosphate were 0.02 to 0.04 μM (Table 1). Toxicity to the macrophage hosts, demonstrated by loss of at least one-half of the macrophages from the culture surface, occurred at 5 to 20 μM for these agents (Table 1). Thioformycin B was less active ($ED_{50}$, 3.6 μM) and less toxic than formycin B (Table 1). The $ED_{50}$s for allopurinol ribonucleoside, thiopurinol, and thiopurinol ribonucleoside had to be estimated owing to the flatness of the dose-response curves. The estimated $ED_{50}$ for thiopurinol was similar to that previously found for allopurinol (40 μM; 4) and the value for thiopurinol ribonucleoside was similar to that for allopurinol ribonucleoside (Table 1). No more than 60% of organisms were eliminated by these drugs at the highest concentrations tested (72 to 190 μM).

The $ED_{50}$ for 7-deazainosine was low (0.2 μM), but this analog eliminated only 80% of organisms at the highest nontoxic concentration (4 μM) (Table 1). The thio derivative of 7-deazainosine was both less active against *Leishmania* and less toxic to macrophages than 7-deazainosine itself (Table 1).

3-Deazaguanosine demonstrated an $ED_{50}$ of 3.6 μM. The $ED_{50}$ of 3-deazaguanine was greater than 134 μM; the $ED_{50}$ for 3-deazaadenosine was greater than 72 μM (Table 1).

## DISCUSSION

Formycin B, formycin A, formycin B monophosphate, formycin A monophosphate, and formycin A triphosphate were the most active agents tested in vitro against *L. tropica*-infected human macrophages and had favorable therapeutic-toxic ratios. The apparent mechanism of action of formycin B is that it is metabolized to formycin B monophosphate, formycin A monophosphate, and formycin A triphosphate by the organisms, which then incorporate the triphosphate into RNA (12, 14). Such metabolism exposes the organisms to potentially toxic formycin A nucleotides and formycin A containing RNA. Since formycin A is generally metabolized by cells to formycin A nucleotides, it is reasonable that both nucleosides display antileishmanial activity. The similarity of the activities suggests that in this model phosphorylation and amination of formycin B is as efficient as phosphorylation of formycin A. The antileishmanial activity of the formycin nucleotides might seem surprising since these phosphates should not be able to pass through macrophage membranes to attack the organisms. However,

TABLE 1. Antileishmanial activity of purine analogs[a]

| Compound No.[b] | Compound Common name | $ED_{50}$ (μM) | ≥90% Effective dose (μM) | Highest dose tested (μM) |
|---|---|---|---|---|
| 1 | Formycin B | 0.04 (0.04–0.04) | 0.20 | 20 (T)[c] |
| 2 | Formycin A | 0.04 (0.04–0.04) | 0.20 | 20 (T) |
| 3 | Formycin B 5′-monophosphate | 0.02 (0.01–0.03) | 0.33 | 5 (T) |
| 4 | Formycin A 5′-monophosphate | 0.03 (0.02–0.04) | 0.50 | 10 (T) |
| 5 | Formycin A 5′-triphosphate | 0.03 (0.02–0.04) | 0.21 | 5 (T) |
| 6 | Thioformycin B | 3.6 | 18 | 18 |
| 7 | Allopurinol ribonucleoside | 76–190 | | 190 |
| 8 | Thiopurinol | 36–142 | | 142 |
| 9 | Thiopurinol ribonucleoside | 72 | | 72 |
| 10 | 7-Deazainosine | 0.2 | >4.0 | 20 (T) |
| 11 | Thio 7-deazainosine | >18 | | 18 |
| 12 | 3-Deazaguanosine | 3.6 | >72 | 72 |
| 13 | 3-Deazaguanine | >134 | | 134 |
| 14 | 3-Deaza-2′-deoxyguanosine | 72 | | 72 |
| 15 | 3-Deazaadenosine | >72 | | 72 |

[a] The percentage of macrophage-contained *Leishmania* spp. surviving exposure to several doses of a purine analog was determined in two to five experiments for each analog. The number of organisms per 100 macrophages in non-drug-treated controls was 805 ± 224 ($n$ = 25). Formycin B or allopurinol was used as a positive control in each experiment. For compounds 1 through 5, for which the dose-response curves were steep, $ED_{50}$s were calculated for each experiment, and the means of these calculated values are listed with the ranges in parentheses. For compounds 6 through 15, for which the dose-response curves were relatively flat, the $ED_{50}$s were estimated by inspection of the data. For compounds for which doses higher than the $ED_{50}$s were tested, the dose that eliminated ≥90% of organisms (≥90% effective dose) in all experiments is listed.

[b] Corresponds to number of structure in Fig. 1.

[c] T, Toxic to macrophages (≥50% loss of macrophages in all experiments).

macrophage plasma membranes have an externally directed 5'-nucleotidase active against adenosine 5'-monophosphate (8), and the formycin 5'-phosphates may be cleaved to the corresponding formycin nucleosides by this enzyme.

The similar antileishmanial activity of allopurinol to thiopurinol and of allopurinol ribonucleoside to thiopurinol ribonucleoside in this model confirms the data from infected tumor macrophages (11). However, the absolute values of activity in this model differed from those in the tumor model. Here, the doses needed to eliminate 90% of *L. tropica* were greater than the highest concentrations tested (72 to 190 μM); in the tumor model, the $ED_{90}$s against *Leishmania donovani* were 37 to 63 μM. The activity of the thio derivatives of formycin B and of 7-deazainosine have apparently not been reported. Both these compounds were much less active and much less toxic than their respective parent compounds in the human macrophage model.

The vastly increased antileishmanial activity of formycin B compared with allopurinol ribonucleoside indicates that *L. tropica* amastigotes within human macrophages clearly distinguish these two close analogs of inosine. Although formycin B has a favorable therapeutic-toxic ratio in this in vitro model, the drug nevertheless was more toxic than allopurinol ribonucleoside to the macrophage hosts, and simultaneous in vivo trial will be necessary to determine which inosine analog has the most in vivo utility. Amastigotes in this model do not distinguish between allopurinol ribonucleoside and thiopurinol ribonucleoside, but the organisms do distinguish formycin B from thioformycin B and 7-deazainosine from thio 7-deazainosine. Macrophage toxicity paralleled antileishmanial activity, however, and the differential activity of these purine analogs compared with their thio derivatives might not be clinically exploitable.

3-Deazaguanosine was more active than allopurinol or allopurinol ribonucleoside in this model. Both the ribose and the precise structure of the base are apparently necessary for maximum in vitro activity of 3-deazaguanosine since its $ED_{50}$ was at least 20 times greater than those of 3-deazaguanine, 3-deaza-2'-deoxyguanosine, and 3-deazaadenosine. In vivo antiviral experiments showed that 3-deazaguanosine and 3-deazaguanine possessed approximately equal activity (1). Guanosine derivatives have not previously been reported to have antileishmanial activity, but the relatively high activity of 3-deazaguanosine in this study suggests that such compounds may have promise as antileishmanial agents.

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