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Activity of Purine Analogs against *Leishmania donovani* In Vivo

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The dose of orally administered 9-deazainosine calculated to suppress 50% of *Leishmania donovani* amastigotes in hamster livers was 19 mg/kg (body weight) per day; 96 to 99% of *Leishmania* organisms were eliminated from the liver and spleen of squirrel monkeys by 50 mg/kg per day. Because these activities were greater than that of the experimental clinical agent allopurinol and comparable to that of the classical agent parenteral pentavalent antimony, 9-deazainosine should be considered for clinical development for visceral leishmaniasis.

Visceral leishmaniasis results from parasitization of the macrophages of the liver and other components of the visceral monocyte-phagocyte system by *Leishmania donovani*. Although parenteral administration of pentavalent antimony (Sb) complexed to carbohydrates in the form of sodium stibogluconate (Pentostam) or meglumine antimonate (Glucantime) is generally effective, the desirability of an orally administrable antileishmanial agent has led to intense interest in the antileishmanial potential of purine analogs, such as allopurinol and allopurinol ribonucleoside, which are apparently specifically metabolized by the parasites (reviewed in reference 8).

Recent in vitro work by Marr and co-workers indicated that the inosine analog 9-deazainosine might be more active but not markedly more toxic than allopurinol ribonucleoside and, presumably, allopurinol itself against *L. donovani* (9). These observations and the need to test antileishmanial purines against *L. donovani* in vivo led to the determination of the activity of such compounds against *L. donovani* in hamsters and squirrel monkeys.

The activity of antileishmanial drugs against *L. donovani*-infected hamsters was determined as previously described (4). In brief, 60- to 80-g male hamsters (*Mesocricetus auratus*) were infected via intracardiac injection with approximately 10^7 *L. donovani* amastigotes (Khartoum strain, WR 378). On days 3 to 6 after infection, groups of animals (six to eight hamsters each) were given hydroxyethylcellulose-Tween 80 (vehicle controls) or meglumine antimonate (positive controls) via the intramuscular route or purine analogs dissolved in vehicle via the oral route. The hamsters were killed 1 day after the last administration of drug, i.e., 1 week after infection, and the number of parasites per liver cell nucleus was determined from Giemsa-stained impression smears. This number was multiplied by the approximate number of liver nuclei per milligram of liver (200,000; reference 4) and by the weight of the liver to calculate the total number of parasites per liver.

The activity of meglumine antimonate and 9-deazainosine against *L. donovani*-infected squirrel monkeys (*Saimiri sciureus*) was determined as recently described (7). Fifteen male monkeys weighing approximately 700 g were injected intravenously with approximately 10^8 *L. donovani* amastigotes obtained from infected hamster spleens. Infected monkeys were given saline or meglumine antimonate (52 or 13 mg of Sb per kg [body weight] per day) via the intramuscular route or 9-deazainosine (200 or 50 mg/kg per day) via the oral

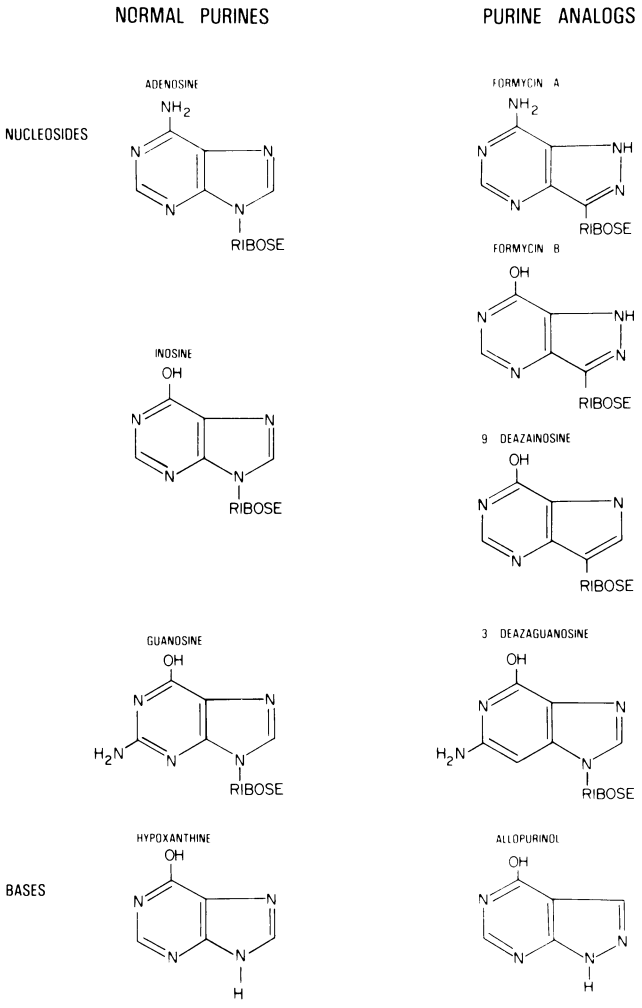

FIG. 1. Structures of purines and purine analogs.

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TABLE 1. Activity of purine analogs against *L. donovani* in hamsters[a]

Drug	Dosage (mg/kg per day)	Mean % wt change	Mean % control liver amastigotes ± SE	ED_{50} (mg/kg per day)	G
Meglumine antimonate	104	−0.6	2 ± 1	13	1
	13	−1.6	49 ± 5		
	3.25	−8.2	79 ± 5		
Allopurinol	208	−2.9	51 ± 2	208	0.06
	52	−6.4	75 ± 1		
	13	−1.2	88 ± 1		
3-Deazaguanosine	208	−3.6	19 ± 2	33	0.39
	52	−4.6	40 ± 3		
	13	+6.0	60 ± 6		
9-Deazainosine					
Expt 1	945	−9.1	0.3 ± 0.1	19	0.68
	416	−11.6	1 ± 0.2		
	208	−6.9	3 ± 1		
Expt 2	208	−8.6	8 ± 1		
	52	−4.0	14 ± 1		
	26	−5.1	26 ± 2		
	6.5	−4.4	89 ± 6		
Formycin A	208	−25.0	6 ± 1	$\ll$13	$\gg$1
	52	−17.9	7 ± 1		
	13	−9.1	14 ± 2		
Formycin B	208	−27.2	2 ± 0.2	$\ll$13	$\gg$1
	52	−16.0	5 ± 0.2		
	13	−8.1	10 ± 1		

[a] Data are values for six to eight hamsters in each group. Control animals had a −3.1% mean weight change and a mean (± standard error) of 576 (± 26) × 10^6 parasites per liver.

route on days 17 to 23 after infection. The monkeys were killed on day 27, and the number of parasites in the liver and spleen was determined as described above. Monkeys given saline or 200 mg of 9-deazainosine per kg per day were necropsied, and all major organs were examined grossly. Selected tissues (liver, spleen, kidney, lung, and brain) were prepared for histologic examination by standard procedures and examined with a light microscope.

For each experimental group, the mean number of parasites in the livers or spleens was determined and expressed as a percentage of the mean number of parasites per liver or spleen in controls. The ED_{50} (dosage of drug expected to suppress 50% of amastigotes in the liver as compared with controls) was approximated by inspection of the data. The G value is the ratio of the ED_{50} of the positive control drug meglumine antimonate to the ED_{50} of the test drug and represents the fold increase in activity of the drug relative to that of meglumine antimonate.

9-Deazainosine was synthesized by the method of Lim et al. (5, 6); the crystalline hydrochloride salt was analytically pure. Allopurinol was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Formycin A and formycin B were obtained from Calbiochem-Behring, La Jolla, Calif. 3-Deazaguanosine was prepared by R. Robins (3). Meglumine antimonate (Glucantime) was purchased from Rhone-Poulenc, New York, N.Y. All other drugs were obtained from the Walter Reed Army Institute of Research drug inventory. Weight of meglumine antimonate refers to weight of pentavalent antimony in the form of meglumine antimonate; meglumine antimonate is approximately 30% antimony by weight.

The activity of purine analogs (Fig. 1) against *L. donovani*-infected hamsters is shown in Table 1. The rank order of in vivo activity was allopurinol (G, 0.06) < 3-deazaguanosine (G, 0.39) < 9-deazainosine (G, 0.68) < formycin A = formycin B (G, >>1). The high in vivo activity of formycin B against visceral leishmaniasis was previously reported for this hamster model (G, 4; reference 2) and for mice

TABLE 2. Activity of 9-deazainosine against *L. donovani* in squirrel monkeys[a]

Drug	Dosage (mg/kg per day)	% Wt change	Mean % (range) control	
			Liver amastigotes	Spleen amastigotes
Meglumine antimonate	104[b]	1.0[b]	2[b] (0–2)	1[b] (0–1)
	52	6.8	6 (0–10)	2 (0–6)
	13	0.3	67 (2–100)	34 (20–94)
9-Deazainosine	200	1.0	1 (0–1)	3 (0–5)
	50	2.6	1 (0–1)	4 (1–7)

[a] Data are values for three monkeys in each group. Control animals had a 5.8% mean weight gain and a mean (range) of 856 (321 to 1,555) × 10^6 parasites per liver and 22 (2 to 59) × 10^6 parasites per spleen.

[b] Previous monkey experiment (1).

(formycin B was 79 times as active as sodium stibogluconate; 10). The rank order of activity of these agents against *Leishmania tropica* (now called "*L. major*") amastigotes in human monocyte-derived macrophages was: allopurinol (ED_{50}, 40 μM) < 3-deazaguanosine (ED_{50}, 3.0 μM) < 9-deazainosine (ED_{50}, 1.0 μM) < formycin A = formycin B (ED_{50}, 0.04 μM) (3; J.D.B., unpublished observations). Although the in vivo and in vitro models use different organisms, the rank order of activity of these agents was the same in the two models, and for some of the drugs the G value ratios were comparable to the ratios of the in vitro ED_{50}s. The comparable data from in vitro and in vivo tests in these two models suggest that the activity of novel purines might be appropriately determined by the use of these two models with a formycin and with allopurinol as strongly active and weakly active control compounds.

9-Deazainosine was the only purine of which there was a dosage that eliminated >95% of parasites (i.e., that was essentially curative) and that did not also result in >>10% mean weight loss, which we arbitrarily chose as an indication of drug toxicity. 9-Deazainosine therefore underwent secondary testing against *L. donovani* in squirrel monkeys. Less than 2% of liver and splenic parasites survived treatment with 104 mg of meglumine antimonate per kg per day, and 2 to 6% of parasites survived 52 mg of meglumine antimonate per kg per day (Table 2). Less than 1% of liver parasites and about 4% of splenic parasites survived treatment with both the high dose (200 mg/kg per day) and the low dose (50 mg/kg per day) of 9-deazainosine. These data suggest that the activity of 50 mg of 9-deazainosine per kg per day is equivalent to that of 52 to 104 mg of meglumine antimonate per kg per day. The only abnormalities observed at necropsy were fatty livers, which were noted in all three monkeys given 200 mg of 9-deazainosine per kg per day and in one of three vehicle control animals. The presence of fatty livers in the monkeys given high-dose 9-deazainosine was verified by light microscopy. There was no histopathologic sign of toxicity in the kidneys, heart, brain, or lungs in this group.

Marr and co-workers found that 9-deazainosine had the most favorable ratio of leishmaniacidal dose-cell toxic dose in vitro (9). Here, 9-deazainosine had the most favorable therapeutic index in *L. donovani*-infected hamsters. 9-Deazainosine was at least as active as meglumine antimonate in *L. donovani*-infected monkeys. In contrast to the virtual elimination of hepatosplenic parasites by 50 mg of 9-deazainosine per kg per day for 7 days, allopurinol ribonucleoside at a dosage of 400 mg/kg per day for 10 days was not significantly active in *Aotus trivirgatus* (W.L.H., unpublished data). We know of no other purine analogs that have been tested in *L. donovani*-infected nonhuman primates. The appearance of fatty livers without abnormalities in other examined tissues in all monkeys given high-dose 9-deazainosine suggests that the liver is the target organ for 9-deazainosine toxicity.

The demonstration that 9-deazainosine virtually eliminates hepatic parasites in *L. donovani*-infected hamsters and at 50 mg/kg per day for 7 days also virtually eliminates hepatosplenic *Leishmania* organisms in *L. donovani*-infected squirrel monkeys suggests that consideration be given to its development as an oral treatment for human visceral leishmaniasis.

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