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Berberine Derivatives as Antileishmanial Drugs

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Berberine, a quaternary alkaloid, and several of its derivatives were tested for efficacy against *Leishmania donovani* and *Leishmania braziliensis panamensis* in golden hamsters. Tetrahydroberberine was less toxic and more potent than berberine against *L. donovani* but was not as potent as meglumine antimonate (Glucantime), a standard drug for the treatment of leishmaniasis. Only berberine and 8-cyanodihydroberberine showed significant activity (>50% suppression of lesion size) against *L. braziliensis panamensis*.

Visceral and cutaneous leishmaniases are endemic diseases in broad areas of the tropics and subtropics. Currently, the mainstay for the therapy of these infections is pentavalent antimony (1), although other drugs, such as pentamidine or amphotericin B, have been used when treatment with antimonial agents has been unsuccessful (10). The relatively low therapeutic indices of these drugs and the fact that they are usually administered under controlled clinical conditions (15) justify continued investigation of alternative compounds for the chemotherapy of leishmaniasis.

Berberine, a quaternary alkaloid widely distributed in nine plant families (14), has demonstrated experimental and clinical efficacies against both visceral (3, 6, 7) and cutaneous (3, 4, 9, 13) leishmaniases. Despite the apparent potential of this compound as an antileishmanial drug, only Putzer (11) has described the antileishmanial activity of derivatives of berberine, and he presented no biologic or chemical data to substantiate his claims. We therefore wished to determine the in vivo antileishmanial properties of several simple analogs of berberine as a preliminary step in establishing a range-finding structure-activity relationship study.

The structures of compounds used in this study are shown in Fig. 1. Berberine chloride (structure 1) was purchased from Aldrich Chemical Co. Palmatine chloride (structure 2) was isolated from *Enantia chlorantha*, and derivatives 3 to 9 were synthesized from berberine as previously described (14).

The activities of the compounds against *Leishmania donovani*, the organism responsible for visceral leishmaniasis, were determined as previously described (8). Briefly, 50- to 70-g golden hamsters (*Mesocricetus auratus*) were infected intracardially with 10^7 spleen-derived amastigotes of a Khartoum strain of *L. donovani*. On day 3 following infection, animals were randomly divided into experimental groups, initial group weights were determined, and drug therapy was initiated and continued through day 6. On day 7 following infection, final group weights were determined, all animals were killed, their livers were removed and weighed, and liver impressions were made for the enumeration of amastigotes. Subsequently, the total number of amastigotes per liver was determined as described by Stauber et al. (12).

The activities of the compounds against *Leishmania braziliensis panamensis*, the etiologic agent of some cases of New World cutaneous leishmaniasis, were determined after intradermal inoculation of hamsters in the area of the depilated dorsal tail head with 1.5×10^7 culture-derived promastigotes of a Panama isolate of this parasite species. On day 19 postinfection, initial group weights and lesion sizes were determined, and treatment was begun and continued through day 22. On day 29 postinfection, final group weights and lesion sizes were determined. Lesion area was calculated from the formula $A = r1r2\pi$, where $r1$ is the major radius of the lesion and $r2$ is the minor radius of the lesion.

Test groups consisted of six hamsters, each of which was treated twice daily over a 4-day period by intramuscular injection of test compounds dissolved in 0.5% hydroxyethylcellulose–Tween 80. The doses used (see Tables 1 and 2) represented the total quantity of compound (on a milligram-per-kilogram body-weight basis) administered to each animal over the entire treatment period. Control groups received 0.5% hydroxyethylcellulose–Tween 80 only. The mean number of parasites per liver (visceral infection) or mean lesion area (cutaneous infection) was determined for test groups, and the percent suppression of parasite burden or lesion area resulting from treatment was calculated by comparison with the mean parasite number or mean lesion area in untreated controls. For purposes of comparing the efficacies of the compounds, suppression of hepatic parasite burden or cutaneous lesion area by approximately 50% or more was considered significant. Death or a loss of >15% of initial group body weight was considered indicative of toxicity.

The activities of berberine and some of its derivatives against visceral leishmaniasis caused by *L. donovani* are shown in Table 1. Only 8-cyanodihydroberberine (structure 5) at a total dose of 208 mg/kg and tetrahydroberberine (structure 7) and *N*-methyltetrahydroberberinium iodide (structure 8), both at 416 mg/kg, suppressed parasite burdens by 50% or more. Among these compounds, 8-cyanodihydroberberine appeared to be the most toxic, as evidenced by a loss of 18% of total group body weight; however, the 11% weight loss in the group treated with *N*-methyltetrahydroberberinium iodide suggests that this compound is more toxic than the virtually equally antiparasitic tetrahydroberberine. None of the compounds showed 50% suppressive activity at 52 mg/kg. In contrast, the reference antileishmanial drug, meglumine antimonate (Glucantime), suppressed parasite numbers by 72% at this dose level. Therefore, tetrahydroberberine, the most potent and least toxic of the derivatives

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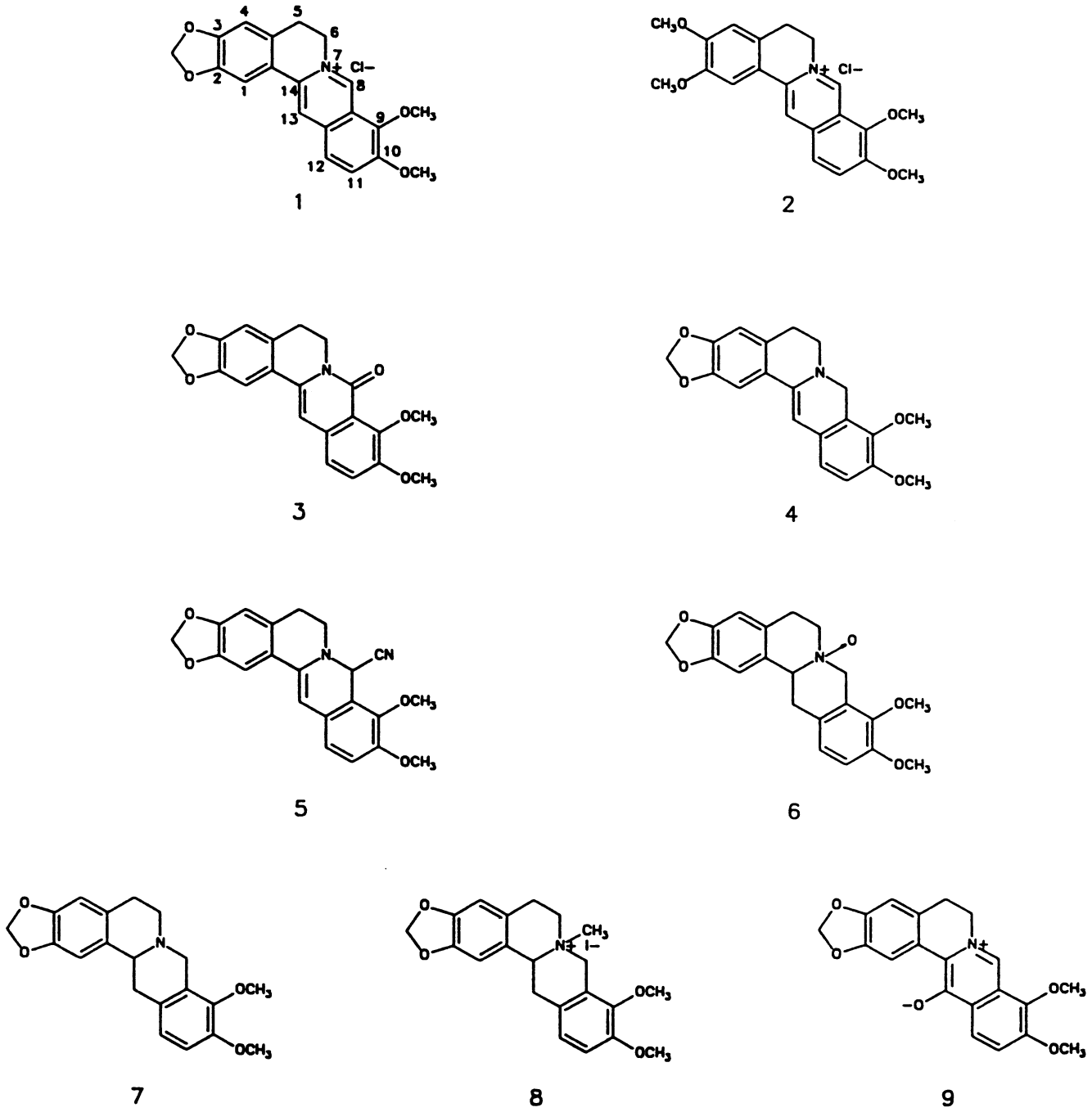

FIG. 1. Structures of berberine alkaloids. 1, Berberine chloride; 2, palmatine chloride; 3, oxyberberine; 4, dihydroberberine; 5, 8-cyanodihydroberberine; 6, tetrahydroberberine *N*-oxide; 7, tetrahydroberberine; 8, *N*-methyltetrahydroberberinium iodide; 9, berberine betaine.

tested, was found to be less effective than the standard pentavalent antimonial drug.

The data in Table 2 represent the percent reduction in lesion area in hamsters infected with *L. braziliensis panamensis*. Inhibition of an increase in lesion size is attributable to the parasiticidal activities of the test compounds, rather than to their effects upon the healing process in this test system (W. L. Hanson, submitted for publication). At the indicated doses, all of the berberine analogs were relatively nontoxic and, with the exception of berberine itself, appeared to be less effective (structures 2, 4, 7, 8, and 9) or equipotent (structures 3, 5, and 6) in the cutaneous test system as compared with the visceral test system. The two most active compounds, berberine and 8-cyanodihydroberberine, produced 56 and 46% suppression of lesion area, respectively, when administered at a total dose of 208 mg/kg. In comparison, meglumine antimonate suppressed lesion development by 66% at the same dose level. These data

TABLE 1. Effect of berberine analogs on numbers of amastigotes in livers of hamsters infected with *L. donovani*

Compound (structure)	Dose (mg/kg)[a]	Suppression (%)[b]	% Wt change[b]
Berberine chloride (1)	52	20	+5
	208[c]	36	−10
Palmatine chloride (2)	52	14	0
	416	28	−11
Oxyberberine (3)	52	0	+9
	416	27	+6
Dihydroberberine (4)	52	23	+7
	416	34	+9
8-Cyanodihydroberberine (5)	52	22	+8
	208[c]	54	−18
Tetrahydroberberine *N*-oxide (6)	52	2	+8
	416	13	+11
Tetrahydroberberine (7)	52	17	+9
	416	50	+7
N-Methyltetrahydroberberinium iodide (8)	52	10	+9
	416	56	−11
Berberine betaine (9)	52	15	+8
	416	23	−11
Meglumine antimonate[d]	52	72	+10
	208	84	+11

[a] Total dose administered over a 4-day period.
[b] As compared with data for animals receiving the 0.5% hydroxyethylcellulose–Tween 80 vehicle only; each treatment group included six hamsters, and seven hamsters were included in each control group.
[c] Compounds were administered at a maximum total dose of 208 mg/kg because of deaths among groups treated with 416 mg/kg in preliminary experiments.
[d] Positive control.

TABLE 2. Effect of berberine analogs on lesion sizes in hamsters infected with *L. braziliensis panamensis*

Compound (structure)	Dose (mg/kg)[a]	Suppression (%)[b]	% Wt change[b]
Berberine chloride (1)	52	22	−2
	208	56	−1
Palmatine chloride (2)	52	0	0
	208	0	−6
Oxyberberine (3)	52	0	+15
	208	21	0
Dihydroberberine (4)	52	0	+1
	208	3	−1
8-Cyanodihydroberberine (5)	52	39	−1
	208	46	−6
Tetrahydroberberine *N*-oxide (6)	52	8	+1
	208	11	−1
Tetrahydroberberine (7)	52	0	0
	208	26	−1
N-Methyltetrahydroberberinium iodide (8)	52	0	0
	208	8	−5
Berberine betaine (9)	52	11	+2
	208	5	−5
Meglumine antimonate[c]	52	22	+2
	208	66	−3

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.
[c] Positive control.

suggest that berberine and 8-cyanohydroberberine are approximately as effective as meglumine antimonate against *L. braziliensis panamensis* in our model.

Because of deaths among animals receiving berberine at 416 mg/kg in preliminary experiments, it was not possible to directly compare the efficacy of the parent compound with those of its derivatives administered at this dose level; therefore, structure-activity correlations must be made with caution. Against *L. donovani* the best activity at 416 mg/kg was observed with tetrahydroberberine and *N*-methyltetrahydroberberinium iodide, both having a tetrahydroberberine skeleton. The weight loss associated with quaternary *N*-methyltetrahydroberberinium iodide suggests that this compound is more toxic than the equally parasiticidal tetrahydroberberine. It would thus appear that the further investigation of tetrahydroberberine derivatives without a quaternary nitrogen atom for the treatment of visceral leishmaniasis is warranted. Against the cutaneous parasite, *L. braziliensis panamensis*, berberine and 8-cyanodihydroberberine were the only compounds that produced significant (ca. 50%) suppression of lesion development. The activity of 8-cyanodihydroberberine may have resulted in part from its oxidation to the corresponding quaternary structure; an analogous oxidation has been reported for dihydroberberine (structure 4) (2). Another quaternary ammonium compound, methylbenzethonium chloride, has demonstrated activity against Old World (*Leishmania major*) cutaneous leishmaniasis (5), suggesting that a quaternary nitrogen atom is a necessary pharmacophore in alkaloids useful against cutaneous leishmaniasis. Surprisingly, however, palmatine chloride (structure 2), a quaternary derivative which differs from berberine only in the substitution of a methylenedioxy group for a bis-methoxy group at the 2,3 position, was inactive.

Variations between visceral and cutaneous test systems in weight changes resulting from drug administration are likely attributable to differences in host age. Although hamsters in both models were approximately the same age when infected, treatment of *L. donovani*-infected hamsters was begun 3 days postinfection, whereas treatment of animals infected with *L. braziliensis panamensis* was delayed until 19 days postinfection, at which time lesions were sufficiently developed for testing. Consequently, treatment of *L. donovani*-infected animals occurred at a time when animals were undergoing a period of growth and were apparently more susceptible to treatment-associated weight loss or growth inhibition. Conclusions regarding the toxicity of a compound based solely upon weight loss are not absolute, but this parameter is a useful indicator of the relative toxicities of compounds within the same model system.

Variations in the susceptibilities of *L. donovani* and *L. braziliensis panamensis* to berberine derivatives may have resulted from differences in parasite susceptibility; however, differences in the delivery of the drugs to the sites of infection (liver versus skin) after intramuscular injection

may have been as important as differences in parasite susceptibility or intrinsic drug activity in contributing to these variations. Reported clinical success against Old World cutaneous leishmaniasis was achieved by local injection of berberine near the site of the lesion (3, 4, 9, 13); however, these studies involved a different *Leishmania* species. To our knowledge, this is the first reported study of the efficacy of berberine against the New World parasite, *L. braziliensis panamensis*. The activities of berberine and its derivatives against this species might improve with injection near the lesion site; however, the association of the *L. braziliensis* complex with disseminated disease might make local, rather than systemic, therapy unacceptable for clinical use.

Although it is not possible to draw definitive conclusions from these preliminary data, certain trends are discernible. With this initial structure-activity relationship information in hand, we hope to be able to more effectively synthesize and test berberine derivatives with increased efficacies for the treatment of leishmaniasis.

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