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Chemotherapy of *Babesia microti* Infections in Mongolian Jirds

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For identifying drugs which might be effective in the treatment of human *Babesia microti* infections, 20 selected antiprotozoal agents or combinations of agents were tested for activity against *B. microti* in Mongolian jirds (*Meriones unguiculatus*). 4-Methyl primaquine and aromatic diamidines, including diminazene and pentamidine, were the most effective compounds tested.

Babesiosis is a tick-borne disease of wild and domestic animals which is occasionally transmitted to humans. It is caused by intraerythrocytic protozoan parasites of the genus *Babesia*. The majority of reported cases of human babesiosis have been caused by *Babesia microti*, a parasite of rodents. Because of morphological similarities between *B. microti* and malaria parasites, most of the patients infected with this organism were treated with oral chloroquine phosphate (3, 4, 6, 7). However, chloroquine appears to be ineffective against *B. microti* infections in animals, and in many of the human patients, it produced symptomatic improvement but had no effect on the course of their parasitemia (3, 4, 6, 7). We attempted, therefore, to identify an effective antibabesial agent among the antiprotozoal drugs currently licensed for use in humans in the United States. *B. microti* infection in the Mongolian jird (*Meriones unguiculatus*) was selected as the test system, because in that host a reproducible parasitemia results after intraperitoneal administration of the parasites. While these studies were in progress, a similar drug screening method with golden hamsters was reported (3).

MATERIALS AND METHODS

The Briard strain of *B. microti*, originally isolated from a patient with babesiosis, was passaged every 2 weeks in 8-week-old male Mongolian jirds (Tumblebrook Farm, Inc., West Brookfield, Mass.) (4). For drug testing, blood was taken from an infected jird by cardiac puncture with a heparinized syringe. The number of parasites per cubic millimeter of blood was calculated based on an erythrocyte count and a count of the number of parasites per 1,000 erythrocytes on a Giemsa-stained thin blood smear. The infected blood was then diluted with sterile saline to achieve the desired concentration, and animals were inoculated intraperitoneally with 0.1 ml of blood containing 2×10^7 to 4×10^7 organisms.

At 5 days after the inoculation of infected blood, the first dose of drug was administered (day 0). Additional doses of drug were given daily for a total of 5 days. The route of administration was intragastric unless otherwise stated. Generally, three different doses of each drug were tested, with the highest level at 300 mg/kg per day unless toxic at that dose. Six animals were treated at each dose. Drugs were administered as salts, and results are expressed as such. A control group of six animals which received distilled water intragastrically was included with each group of three drugs tested. Thin blood smears were made from each animal before the first dose (day 0), before the fourth dose (day 3), and 24 h after the fifth dose of drug (day 5). The blood smears were stained with Giemsa stain, and the percentage of parasitized erythrocytes was determined. Results are expressed as the percent suppression of parasitemia in test animals as compared with control animals. A total of 20 drugs or drug combinations were tested.

Selected compounds which showed activity against *B. microti* based on the above test were then studied in groups of six animals under a regimen in which drugs were administered daily for 14 days, beginning 5 days after the inoculation of infected blood. Blood smears were taken from each animal on days 0, 4, 9, 14, 18, and 21. Animals which showed no evidence of parasitemia on blood smears taken on day 21 were bled by cardiac puncture into a heparinized syringe, and the blood was then injected intraperitoneally into an uninfected jird. These animals were followed with weekly thin blood smears for 6 weeks for evidence of *B. microti* infection.

RESULTS

Sulfamethoxazole, trimethoprim-sulfamethoxazole, pyrimethamine, pyrimethamine-sulfadoxine, tetracycline, clindamycin, metronidazole, quinacrine dihydrochloride, chloroquine phosphate, and stibogluconate sodium showed only slight activity or no activity against *B. microti* in jirds at the highest nonfatal doses tested (Table 1). Two of the six animals which received chloroquine (300 mg/kg per day) died.

The antimalarial drugs dapsone (100 mg/kg per day), primaquine phosphate (50 mg/kg per

day), and pentaquine phosphate (25 mg/kg per day) were moderately active. An experimental 8-aminoquinoline which has antileishmanial activity, 4-methyl primaquine diphosphate (WR-181 023), was one of the most effective drugs studied. It produced a rapid reduction of *B. microti* parasitemia in doses of 25 and 50 mg/kg per day for 5 days.

Six drugs with known antitrypanosomal activity were tested, including melarsoprol, suramin sodium, pentamidine, diminazene, and two experimental diamidines, 2,5-bis (4-guanylphenyl) furan dihydrochloride (WR-199 385) and 3,4-dimethyl-2,5-bis (4-guanylphenyl) furan dihydrochloride (WR-214 400). Suramin sodium produced only a slight reduction of parasitemia at a dose of 100 mg/kg per day. All six animals which received 300 mg/kg per day died. Pentamidine at a dose of only 10 mg/kg per day produced a moderate reduction in the level of parasitemia. A dose of 50 mg/kg per day was tested as well, but five of the six animals died. Melarsoprol, diminazene, WR-199 385, and WR-214 400 were active in doses of less than 10 mg/kg per day. Toxic effects occurred with several of these drugs at higher doses; melarsoprol (72 mg/kg per day) caused the death of four out of six animals, whereas five out of six animals given diminazene at 100 mg/kg per day died.

Primaquine, 4-methyl primaquine, melarsoprol, pentamidine, and diminazene were tested in the 14-day treatment schedule (Table 2). Pri-

TABLE 1. *Activity of various compounds tested in a 5-day treatment schedule against B. microti in Mongolian jirds*

Compound	Highest NFD[a] tested (mg/kg)	% Suppression of parasitemia at NFD on day	
		3	5
Sulfamethoxazole	300	0	0
Dapsone	100	21.9	39.1
Pyrimethamine	25	0	0
Trimethoprim-sulfamethoxazole	60:300	0	0
Pyrimethamine-sulfadoxine	15:300	0	0
Tetracycline	300	23.2	2.7
Clindamycin	300	0	9.4
Metronidazole	300	3.6	4.9
Quinacrine dihydrochloride	100	0	0
Chloroquine phosphate	100	23.3	0
Pentaquine phosphate	25	77.9	96.4
Primaquine phosphate	50	79.6	96.4
4-Methyl primaquine diphosphate	50	99.2	99.9
Stibogluconate sodium[b]	300	11.2	11.0
Melarsoprol[c]	36	99.5	98.3
Suramin sodium[c]	100	41.3	32.6
Pentamidine isethionate[b]	10	17.9	63.0
Diminazene aceturate[b]	25	91.7	99.6
WR 199 385[b]	100	92.0	99.3
WR 214 400[b]	100	93.9	99.5

[a] NFD, Nonfatal dose.
[b] Subcutaneous route of administration.
[c] Intraperitoneal route of administration.

TABLE 2. *Activity of various compounds tested in a 14-day treatment schedule against B. microti in Mongolian jirds*

Compound	Daily dose (mg/kg)	% Suppression of parasitemia on day					Animals subinoculated at day 21 (no. positive/no. tested)
		4	9	14	18	21	
Primaquine phosphate	20	35.7	0	0	0	0	Not tested
4-Methyl primaquine diphosphate	20	99.9	99.9	99.9	99.9	99.9	6/6
Melarsoprol[a]	18	98.1	44.8	0	0	0	Not tested
Pentamidine isethionate[b]	20	94.6	99.9	—[c]	—	—	Not tested
Diminazene aceturate[b]	20	95.3	99.9	99.9	99.9	99.9	0/6

[a] Intraperitoneal route of administration.
[b] Subcutaneous route of administration.
[c] Only two out of six animals survived to day 14.

maquine phosphate (20 mg/kg per day) showed no activity against *B. microti*. 4-Methyl primaquine diphosphate, administered for 14 days at a dose of 20 mg/kg per day, reduced parasitemia to subpatent levels by day 4, but organisms could still be found when the blood of these animals was inoculated into uninfected jirds on day 21. Melarsoprol (18 mg/kg per day) produced only a transient reduction of parasitemia. Pentamidine at a dose of 20 mg/kg per day rapidly reduced parasitemia; however, drug toxicity was such that only two out of six animals survived 14 days. Both of these animals died before they could be examined for subpatent parasitemia on day 21. Diminazene (20 mg/kg per day) had a similar rapid action and when given for 14 days completely eradicated the infecton in all six animals.

DISCUSSION

Mongolian jirds are useful experimental animals for the preliminary screening of compounds for activity against *B. microti*. The parasitemia which results from a standard parasite inoculum is highly reproducible, and there appears to be little animal-to-animal variation in response to a given dose of drug. In addition, the results obtained with this model agree closely with the results reported for chemotherapeutic trials of *B. microti* infections in golden hamsters (3).

The present assessment of 20 antiprotozoal agents against *B. microti* in jirds has shown that the diamidines and 4-methyl primaquine are the most effective of the drugs tested. Similar results have been reported in studies of pentamidine and diminazine in experimental *B. microti* infections in hamsters (3). The marked increase in activity of 4-methyl primaquine over the parent compound, primaquine, parallels that observed in mice infected with *Leishmania donovani* (2). The increased activity appears to be due to the presence of a methyl group in the 4 position. No evidence was found in the studies reported here to suggest that chloroquine has any activity against *B. microti*.

Because of the lack of an effective drug for the treatment of human *B. microti* infections, further study of the diamidines and 4-methyl primaquine against *B. microti* is warranted in an experimental animal model which more closely resembles infection in humans. Both splenectomized and intact rhesus monkeys are susceptible to *B. microti* and may provide a suitable model for such drug trials (1, 5). Trimethoprim-sulfamethoxazole also deserves further study in a primate model. Atlhough it showed no activity against *B. microti* in jirds and has only slight activity against *Plasmodium* in rodent models, it is an effective antimalarial agent in primates (8).

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