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Evaluation of Selected Antiprotozoal Drugs in the *Babesia microti*-Hamster Model

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The presently used therapy for *Babesia microti* infections, a combination of quinine and clindamycin, does not always result in parasitologic cures. To identify possible alternative chemotherapeutic agents for such infections, we screened, in the hamster-*B. microti* system, 12 antiprotozoal drugs that have either recently been released for human use or were in experimental stages of development at the Walter Reed Army Institute of Research for the treatment of malaria and leishmaniasis. Several well-recognized antimalarial drugs, such as mefloquine, halofantrine, artesunate, and artelenic acid, exhibited little or no effect on parasitemia. Two 8-aminoquinolines, WR006026 [8-(6-diethylaminohexylamino)-6-methoxy-4-methylquinoline dihydrochloride] and WR238605 [8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy-7) quinoline succinate], produced clearance of patent parasitemia. Furthermore, blood from infected hamsters treated with WR238605 via an intramuscular injection failed to infect naive hamsters on subpassage, thus producing a parasitologic cure. These two compounds merit further screening in other systems and may prove useful in treating human babesiosis.

Babesiosis is emerging as an important public health concern due to the potential severity of the infection, the apparent spread in geographic range, and the growing recognition that species other than *Babesia microti* cause infection in humans in the United States (12, 13, 18, 19). While *B. microti* generally causes mild to moderately severe illness (9, 10, 16, 18), severe and occasionally fatal infections with high levels of parasitemia have been reported, particularly among asplenic or immunosuppressed patients (3, 7, 13, 14). The currently recommended therapy for *B. microti* and other *Babesia* infections, a 7- to 10-day course of clindamycin plus quinine, is based on its efficacy in laboratory animals (15, 21) and experience with individual patients (6, 22). While this combination therapy does reduce parasitemia, it does not always eliminate the infection, and some patients experience recrudesences or persistent parasitemia (1, 19). This problem is most common in immunosuppressed patients (3) and in those with other underlying medical conditions (7).

Since the initial experimental trials with clindamycin and quinine, various new antimalarial and antiprotozoal drugs have been licensed for use in humans or are undergoing clinical trials and may be available for use in the near future. The purpose of this study was to evaluate several of these drugs for their activities against acute *B. microti* infections and for their ability to eliminate parasitemia completely.

MATERIALS AND METHODS

The Gray strain of *B. microti*, isolated from a patient in 1969 (5) and maintained at the Centers for Disease Control and Prevention since that time, was passaged in golden Syrian hamsters (*Mesocricetus auratus*). For the purpose of this study, parasitemias were determined by counting the number of infected erythrocytes (RBCs) in 200 RBCs in thin blood films stained with Giemsa. To monitor parasitemia, thin blood smears were prepared from a drop of blood collected from the tip of the tail. For inoculations of test animals, 1 cc or more of blood was collected by cardiac puncture from infected donor animals and the percentage of infected RBCs was determined with a drop of blood to prepare a thin smear. Blood was then diluted 1:5 with RPMI so that the volume to be inoculated could be more accurately measured. Experimental animals were inoculated intraperitoneally with approximately 10^7 *B. microti*-infected RBCs in a volume of 0.5 cc.

Drugs were chosen for screening based on one of two criteria. Several drugs currently in use as antimalarials, such as halofantrine, mefloquine, and the artesunate compounds, were selected, as these have not been tested against *Babesia* species. The other compounds were selected from the Walter Reed Army Institute of Research (WRAIR) screening system because of their demonstrated antiprotozoal activity. Several of these are already in phase I or II clinical trials. We arbitrarily chose not to screen compounds that were not likely to be in human trials in the near future, even if they had demonstrated antiparasitic activity. Drugs were obtained from WRAIR or by prescription from a local pharmacy. Drug treatments were initiated when parasitemia was between 3 and 7% (defined as day 0). All drugs were screened at the highest concentration and for the length of time deemed to be maximally effective yet safely tolerated. Drug efficacy, defined as the mean percent suppression of parasitemia compared with that in nontreated control animals, was assessed at 1, 3, and 7 days following the final treatment dose (posttreatment [PT]) for the preliminary screening studies and at 1, 3, 7, and 14 days PT for all subsequent evaluations. Routes of administration were dependent on each drug. Five animals per group were treated with each drug. For subcutaneous (s.c.) and intramuscular (i.m.) administration, the vehicle was peanut oil; for oral (p.o.) administration, the vehicle was hydroxyethylcellulose 0.5%–Tween 80 (HEC). Control animal groups (n = 5 each) consisted of infected animals receiving either peanut oil, HEC, or no treatment.

Compounds screened for activity against *B. microti* included pentamidine isethionate; floxacrine (WR243251); [8-(6-diethylaminohexylamino)-6-methoxy-4-methylquinoline dihydrochloride] (WR006026); [8-[(4-amino-1-methylbutyl) amino]-2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy-7) quinoline succinate] (WR238605); [1,6-dimethyl-2-(4′-formylphenyl) imidazo[1,2-A] pyridinium guanylhydrazone ditosylate] (WR252163); diamidine [4-(4-amidinophenoxy) benzaldehyde-4′-amidinophenylhydrazone dihydrochloride] (WR245720); artelenic acid (WR155663); halofantrine (WR171669); paromomycin; mefloquine (WR42490); artesunate (WR256283); pyrroloquinazoline (WR228275); a combination of clindamycin and quinine; biguanide (WR250417); and guanylhydrazone (WR249928).

Three compounds that showed the greatest activities in the initial trials, the two 8-aminoquinolines (WR006026 and WR238605) and a pyrroloquinazoline (WR228275), were further investigated to determine the minimum dose for

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TABLE 1. Activities of various compounds against *B. microti* in the golden hamster

Compound	Treatment regimen[a]	Mean % suppression of parasitemia on the following day PT[b]:		
		1	3	7
8-Aminoquinoline (WR006026)	12.5 mg/kg b.i.d. × 4 days p.o.	88	100	100
8-Aminoquinoline (WR238605)	52 mg/kg b.i.d. × 4 days i.m.	88	100	100
Pyrroloquinazoline (WR228275)	6 mg/kg b.i.d. × 3 days p.o.	88	97	99
Diamidine (WR245720)	208 mg/kg b.i.d. × 4 days i.m.	90	96	97
Biguanide (WR250417)	100 mg/kg b.i.d. × 4 days p.o.	91	95	95
Artelenic acid (WR155663)	700 mg/kg × 3 days p.o.	88	93	84
Clindamycin plus quinine	150 mg/kg × 7 days p.o. 250 mg/kg × 7 days p.o.	23	22	70
Paromomycin	100 mg/kg × 10 days i.m.	21	10	13
Artesunate (WR256283)	160 mg/kg × 1 day then 80 mg/kg b.i.d. × 5 days p.o.	0	0	9
Mefloquine (WR142490)	250 mg/kg × 1 day p.o.	38	21	0
Halofantrine (WR171669)	80 mg/kg t.i.d. × 1 day p.o.	27	5	0
Pentamidine isethionate	10 mg/kg × 10 days i.m.	26	26	0
Artesunate (WR256283)	20 mg/kg × 1 day, then 10 mg/kg b.i.d. × 5 days p.o.	6	20	0
Acridinoneimine (WR243251)	100 mg/kg × 1 day p.o.	12	0	0
Guanylhydrazone (WR249928)	60 mg/kg b.i.d. × 4 days p.o.	0	0	0
Pentamidine isethionate	40 mg/kg × 15 days p.o.	0	0	0
Guanylhydrazone (WR252163)	13 mg/kg × 1 day s.c.	0	0	0

[a] t.i.d., three times a day.
[b] Results are expressed as percent suppression of parasitemia in treated animals compared to nontreated controls.

parasite clearance. Pentamidine isethionate and a combination of clindamycin plus quinine were included in these dosing studies for comparison purposes.

To determine if either of the two compounds (WR006026 and WR238605) that showed the most activity against *B. microti* during preliminary studies was capable of eliminating parasitemia (i.e., effecting a parasitologic cure), an additional study was conducted. Ten animals per group were used at each dose for this assessment, and animals with no parasitemia 14 days after the last drug dose were bled by cardiac puncture and 0.5 cc of blood was subpassaged intraperitoneally into naive animals. These animals were monitored weekly by thin blood smears for 6 weeks. Pentamidine isethionate and clindamycin plus quinine were included for comparison.

RESULTS

The results of the initial screening are shown in Table 1. The compounds with the greatest percentage of suppression of parasitemia were the 8-aminoquinolines, WR0060260 and WR238605, both of which showed complete suppression of patent parasitemia at 3 and 7 days PT. Diamidine, pyrroloquinazoline, and biguanide all showed ≥95% suppression of parasitemia at 3 and 7 days PT, and artelenic acid suppressed parasitemia by 84% by 7 days PT. Conversely, pentamidine, floxacrine, mefloquine, halofantrine, artesunate, and guanylhydrazone exhibited no suppression of parasitemia by 7 days PT.

Both of the 8-aminoquinolines (WR006026 and WR238605) and the pyrroloquinazoline (WR228275) were reevaluated at lower doses (Table 2). WR006026 completely suppressed patent parasitemia at 3 and 7 days PT, at 3.13 mg/kg of body weight twice a day (b.i.d.) times 4 days p.o. and at 12.5 mg/kg b.i.d. times 2 days p.o. WR238605 completely suppressed parasitemia at 13 mg/kg b.i.d. times 4 days i.m. at 3 and 7 days PT; and after treatment was reduced to 52 mg/kg b.i.d. times 2 days i.m., parasitemia was suppressed fully by day 7 PT. Pyrrolo-

TABLE 2. Effect of reduced dosing regimens and schedules on the antibabesial efficacy of selected compounds against *B. microti* in the golden hamster

Compound	Treatment regimen	Mean % suppression of parasitemia on the following day PT[a]:		
		1	3	7
8-Aminoquinoline (WR006026)	12.5 mg/kg b.i.d. × 4 days p.o.	100	100	100
	3.13 mg/kg b.i.d. × 4 days p.o.	98	100	100
	0.78 mg/kg b.i.d. × 4 days p.o.	91	99	97
	12.5 mg/kg b.i.d. × 2 days p.o.	90	100	100
8-Aminoquinoline (WR238605)	52 mg/kg b.i.d. × 4 days i.m.	68	100	100
	13 mg/kg b.i.d. × 4 days i.m.	41	99	100
	3.25 mg/kg b.i.d. × 4 days i.m.	73	91	94
	52 mg/kg b.i.d. × 2 days i.m.	52	99	100
Pyrroloquinazoline (WR228275)	6 mg/kg b.i.d. × 3 days p.o.	90	77	76
	1.5 mg/kg b.i.d. × 3 days p.o.	62	24	66
	0.375 mg/kg b.i.d. × 3 days p.o.	1	0	58
	6 mg/kg b.i.d. × 1.5 days p.o.	74	23	0

[a] Results are expressed as percent suppression of parasitemia in treated animals compared to nontreated controls.

TABLE 3. Ability of various compounds to eliminate *B. microti* parasitemia in the golden hamster

Compound	Treatment regimen	Mean % suppression of parasitemia on the following day PT[a]:				No. of negative hamsters after subpassage/total no.
		1	3	7	14	
8-Aminoquinoline (WR006026)	12.5 mg/kg b.i.d. × 4 days p.o.	100	100	100	100	4/9
8-Aminoquinoline (WR238605)	52 mg/kg b.i.d. × 4 days i.m.	>99	100	100	100	8/8
Clindamycin plus quinine	150 mg/kg × 7 days p.o. 250 mg/kg × 7 days p.o.	86	92	90	50	Not determined
Pentamidine isethionate	20 mg/kg × 10 days i.m.	20	6	0	0	Not determined

[a] Results are expressed as percent suppression of parasitemia in treated animals compared to nontreated controls.

quinazoline was less effective in the second trial than in the initial trial; the greatest reduction in parasitemia (76%) occurred at 7 days PT at 6 mg/kg b.i.d. times 3 days p.o.

Both 8-aminoquinoline compounds suppressed parasitemia completely through day 14 PT; clindamycin plus quinine provided about 50% suppression on day 14, and pentamidine isethionate did not provide suppression of parasitemia after day 7 PT (Table 3). When blood was subpassaged from the negative (8-aminoquinoline-treated) animals at day 14 PT, approximately half of the animals receiving blood from the group treated with WR006026 p.o. became parasitemic, but none of the animals receiving blood from the group treated with WR238605 i.m. developed parasitemia in the 6 weeks following subinoculation, indicating that parasitologic cure had been achieved.

DISCUSSION

Treatment of *B. microti* infections has always been problematic, and the need for a better drug has become increasingly urgent in recent years due to its spreading geographic range, the threat of infections in human immunodeficiency virus-AIDS patients, and an apparent increase in the number of infections in patients with other predisposing medical conditions. This situation is further complicated by the occurrence of human infections with species other than *B. microti*, which, in general, have been less responsive to conventional treatment with clindamycin and quinine. The inability to achieve parasitologic cures in patients infected with either *B. microti* or *Babesia* spp. heightens concerns over relapsing infections and the potential for subsequent transmission by blood donation and transfusion.

Because *Babesia* infections in older, splenectomized, and immunocompromised patients tend to be more severe with high levels of parasitemia, a drug is needed that rapidly reduces the level of parasitemia. Furthermore, because these individuals cannot rely on their immune systems to assist in the clearance of parasites, ideally therapy should permanently eliminate parasites from the blood.

Several of the drugs we tested showed rapid suppression and/or clearance of parasites from the blood of infected animals. Both 8-aminoquinolines reduced parasitemia by 88% by 24 h after the first dose and cleared parasitemia by day 3 after treatment, while pyrroloquinazoline, diamidine, and biguanide reduced parasitemia to <95% by day 3. In the dosing studies, WR006026 p.o. appeared to be slightly more effective at lower doses than did WR238605 i.m., although by day 7, animals receiving 3.13 mg/kg b.i.d. times 4 days p.o. and 12.5 mg/kg b.i.d. times 2 days p.o. or 13 mg/kg b.i.d. times 4 days i.m. and 52 mg/kg b.i.d. times 2 days i.m. were free of patent parasitemia following administration by either route. On the other hand, in the subpassage studies, blood drawn from animals that had received WR238605 i.m. did not result in infection in subpassaged animals, and thus the drug appeared to have effected a parasitologic cure. In contrast, blood drawn from about half of the animals that received WR006026 p.o. initiated infection when subpassaged, even though blood films were evaluated as negative. By comparison, in the same system, clindamycin plus quinine resulted in only about a 20% reduction in parasitemia on days 1 to 3 and a maximum reduction of between 70 and 90% by day 7 posttreatment. Since all animals in the clindamycin-plus-quinine treatment groups were still parasitemic at day 7, no subpassage experiments were undertaken.

We chose subpassage of blood as our preferred method to assess parasitological cure. Although this method might not be an absolute measure of parasitological cure, it is considered to be an extremely sensitive method for detecting low-level or subpatent *B. microti* parasitemia and is the method used by most investigators to detect such infections in humans. Splenectomy of the recipient animal does not increase its susceptibility to infection (4), and it is not clear that splenectomy or immunosuppression of treated animals provides any greater sensitivity than blood passage in determining a parasitological cure following treatment.

Inasmuch as several of the drugs we tested were considerably more effective than the currently accepted treatment, they would seem to hold great promise for further testing, particularly given the rapid clearance of patent parasitemia and continued suppression for up to 14 days of follow-up and subpassage of blood to clean animals. Two other drugs that have received attention in experimental studies are azithromycin and atovaquone. Azithromycin, either alone or in combination with quinine, was effective in reducing levels of parasitemia of *B. microti* in hamsters during the period of drug administration, but parasitemia rapidly returned to pretreatment levels when the drug was stopped (21). Atovaquone, a 1,4-hydroxynaphthoquinone that has potent antimalarial activity and activity against *Pneumocystis* and *Toxoplasma* species, was somewhat more effective. Most experimentally infected hamsters survived infection and parasitemia never exceeded 1% in treated animals over 54 days of observation (8). Based on our findings, the two 8-aminoquinolines tested in the present study show even greater activity against *B. microti* than either azithromycin or atovaquone.

As a class, the 8-aminoquinolines have marked activity against *Babesia* species. Primaquine phosphate and 4-methyl primaquine diphosphate (WR181023) were previously shown to be active against *B. microti* in the jird model (17). The present study confirms that 8-aminoquinolines will be an effective class of compounds against *Babesia* organisms. Both WR238605 and WR006026 have been through phase I trials; WR006026 is currently undergoing phase II and III trials for use against *Leishmania* and *Pneumocystis* species, while phase

II trials are underway with WR238605 for use against malaria. Studies are currently being planned to evaluate the efficacy of both WR238605 and WR006026 in a second rodent model, as well as in primates, against *B. microti* and a more pathogenic *Babesia* sp. (WA1), isolated from patients in Washington and California. If we are able to confirm efficacy in these systems, there is a strong likelihood of having either or both of these compounds available in the near future for the treatment of human babesiosis.

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