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Antimalarial Activity of WR 243251, a Dihydroacridinedione

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WR 243251 is a dihydroacridinedione that was evaluated for antimalarial blood schizonticidal activity in vitro and in vivo. The in vitro doses calculated to kill 50% of organisms were 11 nM for a chloroquine-susceptible, mefloquine-resistant standard strain and 25 nM for a chloroquine- and pyrimethamine-resistant standard strain. The total dose needed to cure 100% of mice infected with a drug-susceptible strain of *Plasmodium berghei* was 12 to 20 mg/kg of body weight for both oral and subcutaneous administration. The regimen needed to cure 100% of *Aotus* monkeys infected with *Plasmodium falciparum* was 8 mg/kg/day for 3 days (chloroquine-susceptible strain) and 16 mg/kg/day for 3 days (chloroquine-resistant strain). The 100% curative doses for *Aotus* monkeys did not increase for parasites previously exposed to subcurative doses. The absolute value of the curative doses of WR 243251 was comparable to or lower than the values for clinical antimalarial agents. The high absolute activity, comparability of activities against susceptible and resistant parasites, and inability to induce resistance by exposure to subcurative doses suggest that WR 243251 has strong potential as a blood schizonticidal agent.

There are several major structural classes of drugs used to treat malaria due to *Plasmodium falciparum*. The oldest recognized antimalarial agent (quinine), the antimalarial agents used in World War II (chloroquine and quinacrine), and newer agents used for chloroquine-resistant *P. falciparum* (mefloquine and halofantrine) are amino-substituted or methanol-substituted multiring structures. Two other structural classes, dihydrofolate reductase inhibitors (such as pyrimethamine) and *p*-aminobenzoic acid analogs (such as sulfadoxine), are used in combination, and the newest agents are artemisinin analogs. Nevertheless, *P. falciparum* can be clinically resistant to all these drugs in Southeast Asia; except in the case of the artemisinins, there is generally in vitro resistance as well (2, 4, 11). In Southeast Asia, the combination of quinine and an antibacterial antibiotic, tetracycline, is the treatment of choice for multi-drug-resistant *P. falciparum* infections (10).

Quinacrine is a 9-aminoacridine (Fig. 1) that is now rarely used because of parasite resistance; prominent cutaneous, neuropsychiatric, and gastrointestinal side effects; and a host of milder side reactions (increased gastrointestinal motility, fever, vertigo, sweating, arthralgias [1a]). Attempts at the Walter Reed Army Institute of Research to improve upon quinacrine led to the synthesis of the dihydroacridinedione floxacrine (Fig. 1). However, floxacrine was insufficiently effective in the *P. falciparum*-infected *Aotus* monkey model; an additional problem was that the parasites rapidly became resistant to the drug in this model (7). WR 243251 (Fig. 1) is a close analog of floxacrine. We report here the in vitro and preclinical in vivo antimalarial activities of WR 243251 as a blood schizonticide.

MATERIALS AND METHODS

In vitro experiments. The in vitro model of activity against *P. falciparum* has been previously reported (3). In brief, *P. falciparum* organisms within human erythrocytes in 96-well microtiter plates are exposed to serial dilutions of drug for 48 h. Radioactive hypoxanthine is then added. After a further 24 h, the amount of radiolabel in the cultures, which correlates with the number of *P. falciparum* organisms surviving drug exposure, is determined. Computer analysis of the values of incorporated radiolabel versus drug concentration permits calculation of the drug concentration that would reduce incorporation of radiolabel by 50% in this model (IC_{50}). The *P. falciparum* clones used in this work are our standard clones W2 (resistant to chloroquine and pyrimethamine and susceptible to mefloquine) and D6 (susceptible to chloroquine and pyrimethamine and moderately resistant to mefloquine). In addition, we used more recently obtained isolates: Nigeria 30 (resistant to chloroquine), Nigeria 59 (resistant to mefloquine), and TM91 from Thailand (resistant to chloroquine, quinine, and mefloquine). The Nigeria strains were obtained courtesy of A. Oduola. TM91 was obtained courtesy of D. Kyle.

Rodent experiments. Rodent experiments were performed according to the methods of Rane and Kinnamon (5) and Thompson et al. (9), with modifications. Blood stage parasites of the rodent *Plasmodium* sp., *Plasmodium berghei* (KBG 173 strain), within erythrocytes were obtained from donor Swiss mice. At the beginning of the experiment (day 0), 500,000 parasites were inoculated intraperitoneally into naive mice. In experiments in which drug was administered once, drug was administered on day 3 only. In experiments in which drug was administered multiple times, drug was administered twice a day on days 3, 4, and 5 after parasite inoculation. The rate of survival of the mice on day 60 after parasite inoculation was determined. Control mice (animals given vehicle only) typically die of malaria between days 7 and 30. Mice to which drug is toxic typically die on days 3 to 6.

Simian experiments. Simian experiments were performed according to the methods of Rossan et al. (6). Blood stage parasites of the chloroquine-resistant human *Plasmodium* sp., *P. falciparum* (Vietnam Smith/RE strain) or the chloroquine-susceptible *P. falciparum* (Uganda Palo Alto strain) within erythrocytes were obtained from donor *Aotus l. lemurinis*

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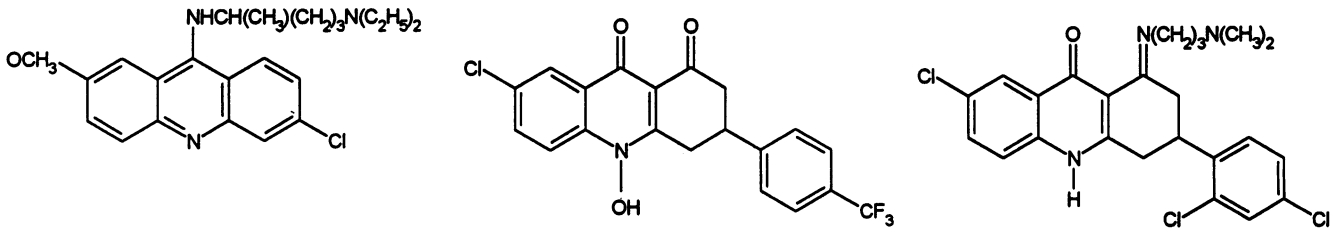

FIG. 1. Chemical structures of antimalarial agents.

monkeys and were injected into malaria-naive monkeys. When the animals had parasitemia levels of approximately 5,000 organisms per mm^3, drug was administered orally once each day for the next 7 days.

Animals were evaluated for parasitemia daily until parasites cleared and then twice weekly until day 100 to determine initial clearance of parasites and recrudescence of parasitemia.

Drugs. All drugs were obtained from the Walter Reed Army Institute of Research drug repository. For oral administration, drug was suspended in 0.3% hydroxymethylcellulose–0.1% Tween 80. For subcutaneous administration, drug was suspended in peanut oil.

RESULTS

In vitro experiments. WR 243251 was approximately 10 times more active than its analog, floxacrine, against both the W2 (chloroquine- and pyrimethamine-resistant) strain and the D6 (moderately mefloquine-resistant) strains of *P. falciparum* (Table 1). As for floxacrine, the activity of WR 243251 differed only by a factor of approximately 2 for the two strains. WR 243251 has one chiral carbon, and the two enantiomers of WR 243251 were separately tested for in vitro efficacy against the W2 strain. WR 243251 was twice as active as the *R* isomer and was much more active than the *S* isomer (Table 1). The values for experiment 2 in Table 1 demonstrate the comparable in vitro efficacies of WR 243251 against strains from several regions of endemicity of the world with different susceptibilities to clinical antimalarial agents.

Rodent experiments. The blood schizonticidal efficacy of single doses of WR 243251 against drug-susceptible *P. berghei* in mice is shown in Table 2. When administered once subcutaneously, WR 243251 was noncurative to slightly curative at a total dose of 5 mg/kg of body weight or less, cured all mice at 20 to 80 mg/kg, and was toxic to approximately half the animals at 640 mg/kg. In comparison, the *R* isomer cured all animals at 160 mg/kg but was toxic to four of five animals at 640 mg/kg; the *S* isomer was inactive at 640 mg/kg (data not shown).

The efficacy of orally administered and parenterally administered WR 243251 was compared in a second experiment (Table 2). The total dose of drug was divided among six administrations. Under these conditions, orally administered drug was as effective as parenterally administered drug (100% curative dose = 12 mg/kg), and the 100% toxic dose was 768 mg/kg.

Simian experiments. When drug was administered daily for 3 days to animals infected with the chloroquine-susceptible (Palo Alto) strain of *P. falciparum*, the dosage needed to cure 50% of animals was 2 mg/kg/day and the dosage needed to cure 100% of animals was 8 mg/kg/day (Table 3). For the chloroquine-resistant (Smith) strain, the 50% curative dose was ~4 mg/kg/day and the 100% curative dose was 16 mg/kg/day.

In the simian model, it is routine to retreat parasites that recrudesce, after exposure to subcurative drug doses, with higher doses of drug. The development of resistance in response to subcurative doses of WR 243251 can be inferred

TABLE 1. Efficacy of WR 243251 against *P. falciparum* strains within human erythrocytes in vitro

Expt	Parasite strain	IC_{50} (nM) of[a]:			
		Floxacrine	WR 243251 (racemic)	WR 250547 (R isomer)	WR 250548 (S isomer)
1	W2	478	25	50	641
	D6	270	11	ND	ND
2	W2	ND	38 (29–55)	ND	ND
	D6	ND	29 (24–42)	ND	ND
	Nigeria 30	ND	17 (17–17)	ND	ND
	Nigeria 59	ND	26 (26–26)	ND	ND
	TM91	ND	51 (44–57)	ND	ND

[a] For experiment 1, data are the IC_{50}s for one experiment; for experiment 2, data are the mean (range) IC_{50}s for two to four experiments. ND, not done.

TABLE 2. Efficacy of WR 243251 against blood stages of *P. berghei* in mice

Expt[a] and route of administration	Total dose (mg/kg)	Survival rate (%)[b]
1, subcutaneous	2.5	0/5 (0)
	5	1/5 (20)
	10	3/5 (60)
	20	5/5 (100)
	40	5/5 (100)
	80	5/5 (100)
	160	4/5–5/5 (80–100)
	640	3/5–5/5 (60–100)
2		
Subcutaneous	3	0/7 (0)
	12	7/7 (100)
	48	7/7 (100)
Oral	3	0/7 (0)
	12	7/7 (100)
	48	7/7 (100)
	192	7/7 (100)
	768	0/7 (0)
	3,072	0/7 (0)

[a] WR 243251 was given once in experiment 1 and twice a day for 3 days in experiment 2.

[b] Number of surviving animals/total number tested.

TABLE 3. Efficacy of WR 243251 against blood stages of *P. falciparum* in *Aotus* monkeys

Parasite strain	Dose (mg/kg/day)[a]	Cure rate (%)[b]
Smith	2	1/4 (25)
	4	5/7 (71)
	8	4/5 (80)
	16	6/6 (100)
Palo Alto	2	2/4 (50)
	4	4/6 (67)
	8	6/6 (100)
	16	4/4 (100)

[a] *P. falciparum*-infected *Aotus* monkeys were administered drug orally once a day for 3 days beginning the day after a parasitemia level of approximately 5,000 organisms per mm^3 was reached.
[b] Number of animals cured/total number tested (cure is defined as clearance of parasitemia with no recrudescence).

from such data. One animal infected with the Palo Alto strain and one infected with the Smith strain were administered a subcurative dosage, 1 mg/kg/day for 3 days, of WR 243251. As expected, there was initial clearance of parasites but also parasite recrudescence (Table 4). After the animals had experienced recrudescence, they were retreated for 3 days with twice the previous daily dose. Examination for recrudescence and further retreatment were continued until the animals were cured. The dosages of drug needed to cure infection with the Palo Alto strain in the fourth course of treatment and the Smith strain in the fifth course of treatment were 8 and 16 mg/kg/day, respectively, the same as the dosage needed to cure infection with these strains in animals not previously exposed to subcurative drug regimens.

DISCUSSION

Clinical resistance of *P. falciparum* to quinacrine and the ability to rapidly create resistance in animals to a dihydroacridinedione, floxacrine, led to attempts to find another analog that was active and against which resistance was unlikely to occur.

WR 243251 is, like floxacrine, a dihydroacridinedione. In this work, we report that against our standard strains (W2 and D6) of *P. falciparum*, the in vitro activity of WR 243251 (IC_{50} = 11 to 25 nM) is approximately 20 times greater than that of floxacrine. The in vitro activity of WR 243251 against the chloroquine-resistant, pyrimethamine-resistant strain W2 was only twofold less than the activity against the susceptible strain D6. In comparison, typical IC_{50}s for other active blood schizonticides against D6 and W2, respectively, are 1.5 and 150 nM for chloroquine, 28 and 101 nM for quinine, 31 and 4 nM for mefloquine, 7 and 2.5 nM for artemisinin, 1 and 1 nM for halofantrine, and 0.2 and 108 nM for pyrimethamine (4a). The in vitro activity of WR 243251 was not diminished when it was tested against chloroquine-, quinine-, or mefloquine-resistant strains more recently obtained from Nigeria or Thailand. The in vitro data therefore suggest that the efficacy of WR 243251 is unlikely to be significantly decreased in chloroquine-, mefloquine-, or pyrimethamine-resistant isolates.

Against blood stages of drug-sensitive *P. berghei* in mice, WR 243251 was 100% curative when administered once subcutaneously at 20 mg/kg. When the drug was given orally or subcutaneously over six administrations, the 100% curative total dose was 12 mg/kg. These values are far lower than historic values for other blood schizonticides: the total oral dose of chloroquine, quinine, mefloquine, halofantrine, artelinic acid, and pyrimethamine needed to cure 100% of animals, when given over six administrations, is >192 mg/kg (1).

In *Aotus* monkeys infected with a drug-susceptible (Palo Alto) strain or a chloroquine-, quinine-, pyrimethamine-resistant (Smith) strain, the 100% curative dosage was 8 or 16 mg/kg/day, respectively, for 3 days. In comparison, the curative dosage for mefloquine is approximately 5 mg/kg/day for 7 days (8). Resistance to WR 243251 did not occur in the Palo Alto or Smith strain in *Aotus* monkeys that were exposed to subcurative doses of drug. After the parasites had recrudesced from several subcurative drug regimens, the curative dose was the same as that needed to cure infections due to parasites never before exposed to drug. This lack of resistance contrasts with apparent creation of resistance to floxacrine due to exposure of *P. falciparum* to subcurative doses of drug. When a naive *Aotus* monkey was infected with the Oak Knoll strain of *P. falciparum* and then administered 40 mg of floxacrine per kg per day for 7 days, parasites were cleared in 6 days, although recrudescence occurred 18 days later. Treatment of another monkey in its first recrudescence with 80 mg/kg/day resulted in parasite clearance in 4 days and no further recrudescence. However, when an infected animal was first treated with 2.5 mg/kg/day and recrudescences were managed by successive doubling of

TABLE 4. Lack of generation of resistance to WR 243251 in *Aotus* monkeys retreated for recrudescence of *P. falciparum* infection[a]

Strain	Treatment course	Pretreatment parasitemia level[b]	Dose (mg/kg/day)[c]	Parasitemia level[b] on posttreatment day:		Result
				1	7	
Uganda Palo Alto	1	40,000	1	37,000	0	Recrudescence
	2	10	2	10	10	Recrudescence
	3	1,000	4	200	0	Recrudescence
	4	15,000	8	100	0	Cure
Vietnam Smith	1	2,000	1	16,000	0	Recrudescence
	2	154,000	2	37,000	0	Recrudescence
	3	38,000	4	60	0	Recrudescence
	4	200	8	0	0	Recrudescence
	5	3,000	16	200	0	Cure

[a] *P. falciparum*-infected monkeys were treated with subcurative doses of WR 243251 and then retreated with twice the previous daily dose when recrudescence occurred.
[b] Expressed as number of organisms per cubic millimeter.
[c] WR 243251 was given daily for 3 days.

the daily dose, a regimen of 80 mg/kg/day did not even clear the blood of parasites (7).

Although there is a large therapeutic index in mice between the minimum dose of WR 243251 needed to cure infection in 100% of animals (20 mg/kg) and the approximate dose needed to kill 50% of animals (640 mg/kg), there will certainly be more subtle side effects that occur at lower doses. By analogy to quinacrine and floxacrine, there is concern about possible dermatologic, cardiac, and neuropsychiatric toxicity and vascular side effects. Nevertheless, the attractiveness of the efficacy data for WR 243251 has caused this compound to enter formal toxicological evaluation and potential clinical development as a blood schizonticidal agent.

REFERENCES

1. **Ager, A., et al.** Unpublished data.

1a. **Anonymous.** 1993. Quinacrine, p. 2136. *In* Physicians' desk reference, 47th ed. Medical Economics Data, Oradell, N.J.

2. **Childs, G. E., E. F. Boudreau, T. Wimonwattratee, L. Pang, and W. K. Milhous.** 1991. In vitro and clinical correlates of mefloquine resistance of Plasmodium falciparum in eastern Thailand. Am. J. Trop. Med. Hyg. **44**:553–559.

3. **Desjardins, R. D., C. J. Canfield, J. D. Haynes, and J. D. Chulay.** 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. **16**:710–718.

4. **Looareesuwan, S., C. Viravan, S. Vanijanonta, P. Wilairatana, P. Suntharasamai, P. Charoenlarp, K. Arnold, D. Kyle, C. Canfield, and K. Webster.** 1992. Randomized trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria. Lancet **339**:821–824.

4a. **Miller, R., et al.** Unpublished data.

5. **Rane, D. S., and K. E. Kinnamon.** 1979. The development of a "high volume tissue schizonticidal drug screen" based upon mortality of mice inoculated with sporozoites of Plasmodium berghei. Am. J. Trop. Med. Hyg. **28**:937–947.

6. **Rossan, R. N., J. S. Harper III, D. E. Davidson, Jr., A. Escajadillo, and H. A. Christensen.** 1985. Comparison of Plasmodium falciparum infections in Panamanian and Colombian owl monkeys. Am. J. Trop. Med. Hyg. **34**:1037–1047.

7. **Schmidt, L. H.** 1979. Antimalarial properties of floxacrine, a dihydroacridinedione derivative. Antimicrob. Agents Chemother. **16**:475–485.

8. **Schmidt, L. H., J. Rasco, and D. Vaughan.** 1978. Antimalarial activities of various 4-quinoline methanols with special attention to WR 142490 (mefloquine). Antimicrob. Agents Chemother. **13**:1011–1030.

9. **Thompson, P. E., B. Olszewski, A. Bayles, and J. A. Waitz.** 1967. Relations among antimalarial drugs: results of studies with cycloquanil-, sulfone-, or chloroquine-resistant Plasmodium berghei in mice. Am. J. Trop. Med. Hyg. **16**:133–145.

10. **Watt, G., L. Loesuttivibool, G. D. Shanks, E. F. Boudreau, A. E. Brown, K. Pavanad, H. K. Webster, and S. Wechgritaya.** 1992. Quinine with tetracycline for the treatment of drug-resistant falciparum malaria in Thailand. Am. J. Trop. Med. Hyg. **47**:108–111.

11. **Webster, H. K., E. F. Boudreau, K. Pavanand, K. Yongvanitchit, and L. W. Pang.** 1985. Antimalarial drug susceptibility testing of Plasmodium falciparum in Thailand using a microdilution radioisotope method. Am. J. Trop. Med. Hyg. **34**:228–235.