

## WR 238605, CHLOROQUINE, AND THEIR COMBINATIONS AS BLOOD SCHIZONTICIDES AGAINST A CHLOROQUINE-RESISTANT STRAIN OF *PLASMODIUM VIVAX* IN *AOTUS* MONKEYS

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**Abstract.** The compound WR 238605 is a primaquine analog being developed by the U.S. Army as an antimalarial drug. Currently, there is no established treatment for *Plasmodium vivax* parasitemias that are not cured by chloroquine. This study tested WR 238605, chloroquine, and their combinations against a chloroquine-resistant strain of *P. vivax* (AMRU 1) in *Aotus* monkeys. A total dose of 3 mg/kg of WR 238605 given at a dosage of 1 mg/kg/day for three days cleared patent parasites in all eight monkeys but recrudescence of parasitemia occurred 15–25 days after initiation of treatment. A total dose of 9 mg/kg of WR 238605 over a three-day period cured all three monkeys of their infections. A total dose of 30 mg/kg of chloroquine did not clear patent infections in three monkeys, whereas a total dose of 60 mg/kg generally (two of three) cleared patent parasitemia but did not cure. Whereas total doses of 30 mg/kg of chloroquine or 3 mg/kg of WR 238605 given alone failed to cure, both drugs given in combination at these dosages cured two of three infections. These results indicate that WR 238605 may be an alternative treatment for chloroquine-resistant vivax malaria.

Some strains of *Plasmodium vivax* from the Melanesian and Indonesian archipelagoes have demonstrated resistance to treatment with chloroquine.<sup>1,2</sup> Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine treatment of vivax malaria. Patients infected with chloroquine-resistant strains of *P. vivax* are treated with repeated courses of chloroquine<sup>1</sup> or mefloquine,<sup>3</sup> but often have multiple recrudescences and/or relapses. Although 8-aminoquinolines, such as primaquine, have primarily been used to eliminate residual liver parasites of relapsing malaria, they also have some activity against the erythrocytic stages.<sup>4</sup> Primaquine has been shown to be an alternative to chloroquine treatment for vivax infections from an area where the parasites are thought to be sensitive to chloroquine.<sup>5</sup> The compound WR 238605 is a primaquine analog developed by the U.S. Army as a better-tolerated, more effective replacement for primaquine. Like primaquine, it is an 8-aminoquinoline with an additional methoxy group at the 2 position, a methyl group at the 4 position, and a 3-trifluoromethylphenoxy substitution at the 5 position of the quinoline ring. Using WR 238605 alone and in combination with chloroquine in owl monkeys (*Aotus lemurinus lemurinus*), we sought to learn if this 8-aminoquinoline could be a potential alternative treatment for chloroquine-resistant vivax infections.

### MATERIALS AND METHODS

The AMRU 1 strain of *P. vivax* was originally obtained from an Australian Special Air Service soldier who was infected in East New Britain Province of Papua New Guinea and failed two sequential treatments with chloroquine.<sup>1</sup> It was subsequently demonstrated that the infection in *Aotus* monkeys could be carried through the mosquito cycle,<sup>6</sup> that high doses of 4-aminoquinolines (60 mg base/kg total dose) were required to cure infections,<sup>7</sup> and that WR 238605 was probably a useful drug in eliminating blood parasites.<sup>8</sup> Using this preliminary information, AMRU 1 was adapted to Panamanian *Aotus* monkeys through > 10 blood passages and

then used in experiments to delineate its sensitivity to WR 238605, chloroquine, and their combinations. Infection was through intravenous inoculations of  $5 \times 10^6$  erythrocytic parasites from a donor monkey. Drug administration followed 5–6 days later when parasitemia reached > 5,000 parasites/mm<sup>3</sup>. Stock solutions of chloroquine were prepared at appropriate concentrations of drug base and maintained at 4°C during the course of treatment. Chloroquine doses are expressed as mg/kg of base drug. A suspension of WR 238605 was prepared in 0.3% methylcellulose just prior to use. Drugs were administered over a three-day period in equally divided doses once a day by gastric intubation in a volume of 7 ml, followed by a 7-ml rinse with either water or 0.3% methylcellulose. Giemsa-stained blood smears were prepared from all animals and examined daily beginning the day after inoculation until parasitemia was cleared and for at least seven days thereafter. Blood films were then examined twice a week up to 100 days after treatment. Blood films were considered negative if no parasites were seen after 5 min of examination. Parasitemia was enumerated by the Earle-Perez technique and expressed as number of parasites/microliter.<sup>9</sup>

All animal experiments were conducted according to the principles enunciated in the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Research, National Research Council, NIH Publication No. 85-23 and applicable federal laws and regulations.

### RESULTS

Infections with the AMRU 1 strain of *P. vivax* were reproducibly resistant to dosages of chloroquine that cured chloroquine-sensitive strains of *P. vivax*. Total doses of 35 mg/kg ( $n = 3$ ) or less of chloroquine did not clear infections (Figure 1 and Table 1). A total dose of chloroquine 60 mg/kg given over three days cleared patent infections in two of three monkeys, but did not produce cures with parasites reappearing by day 10 as shown in Figure 1. Compound WR 238605 cleared patent parasitemias of AMRU 1 when a total

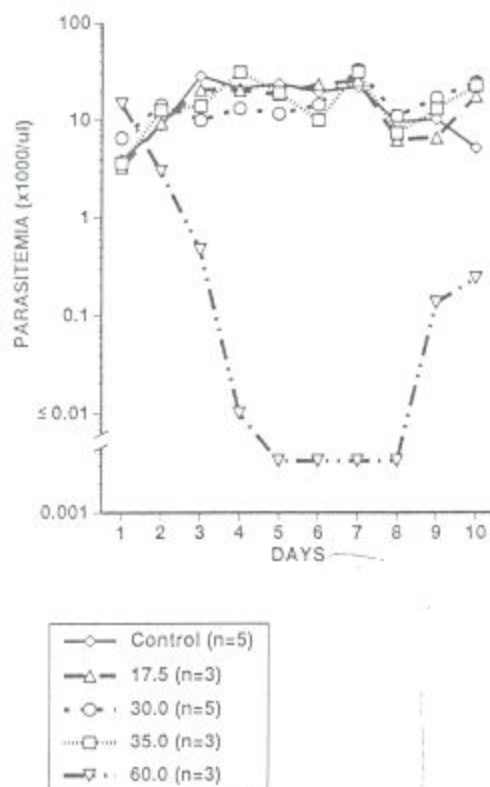


FIGURE 1. Serial parasite counts of chloroquine-resistant *Plasmodium vivax* (AMRU 1)-infected *Aotus* monkeys treated with chloroquine (17.5–60 mg/kg total dose).

dose of 3 mg/kg was given over a three-day period ( $n = 8$ ) as seen in Figure 2, but recrudescences occurred between days 15 and 25. The lowest curative dose of WR 238605 was 9 mg/kg ( $n = 3$ ) as seen in Table 1.

Subcurative doses of chloroquine and WR 238605 were used in combination to treat *Aotus* monkeys infected with the chloroquine-resistant AMRU 1 strain (Figure 2 and Table 1). Each of the combinations tested produced a marked enhancement of activity against the erythrocytic stages when compared with the drugs used alone. For example, a total dose of 0.9 mg/kg of WR 238605 plus 30 mg/kg of chloroquine produced complete clearance of parasitemia in all monkeys tested ( $n = 3$ ), whereas the same dose of WR 238605 alone gave only minor suppression of parasitemia and chloroquine alone had no effect. When examining cured infections, a total dose of 3 mg/kg of WR 238605 plus 30 mg/kg of chloroquine produced cures in two of three animals as opposed to none of eight monkeys cured when only 3 mg/kg of WR 238605 was used.

#### DISCUSSION

The drug WR 238605, an 8-aminoquinoline analog of primaquine, is effective in eliminating the erythrocytic stages of chloroquine-resistant vivax malaria in the *Aotus* monkey. Comparison experiments with primaquine indicate that WR 238605 is approximately 10 times more potent than primaquine as has been seen in previous primate experiments using the rhesus monkey/*P. cynomolgi* model (Heisey G, Armed Forces Research Institute of Medical Sciences, unpublished data). The antimalarial activity of WR 238605 is

TABLE 1  
Effects of WR 238605, alone and in combination with chloroquine, against the AMRU 1 strain of *Plasmodium vivax*\*

Total dose of drug (mg/kg)		Clearance of parasites	Cure of infection
WR 238605	Chloroquine		
—	30	0/5	
—	35	0/3	
—	60	2/3	0/3
0.3	—	0/6	
0.3	30	0/3	
0.9	—	0/6	
0.9	30	3/3	0/3
3	—	8/8	0/8
3	30	3/3	2/3
9	—	3/3	3/3
36	—	3/3	3/3

\* Drug dosages are in mg/kg total dose. Fractions are monkeys cleared or cured over the total number treated. Clearance of parasites means repeated negative daily smears were seen, while cure means that the infection was eradicated over the 100-day observation period.

further enhanced by the addition of subtherapeutic doses of chloroquine. Previous studies with *P. yoelii* in mice suggested that WR 238605 could be synergistically combined with chloroquine against chloroquine-resistant infections.<sup>10</sup> Although the combination of WR 238605 and chloroquine certainly enhanced parasite clearance and cures in this study, the combination appears to be only additive in *Aotus* monkeys.

The data from this study have clinical relevance since potential uses for WR 238605 include the treatment of chloroquine-resistant vivax malaria and terminal prophylaxis of

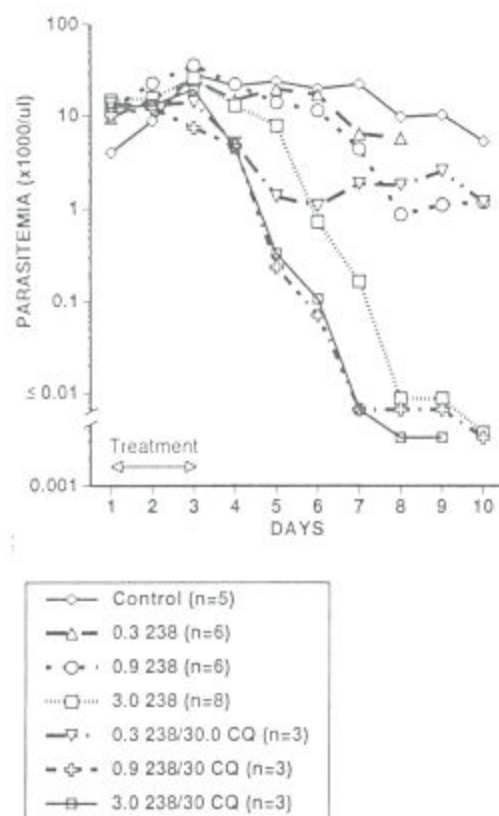


FIGURE 2. Serial parasite counts of chloroquine-resistant *Plasmodium vivax* (AMRU 1)-infected *Aotus* monkeys treated with WR 238605 (0.3–3 mg/kg) alone or in combination with chloroquine (CQ) (30 mg/kg).

travelers to eliminate residual parasites on leaving an endemic area. In most malaria-endemic areas, vivax malaria patients are treated with chloroquine to eliminate erythrocytic stages plus at least two weeks of primaquine to prevent relapses due to persisting exoerythrocytic stages. It may be easier to accomplish the same cure with a short course of chloroquine plus WR 238605. It has been shown that primaquine alone can eliminate erythrocytic stages in vivax malaria although primaquine worked better in combination with chloroquine.<sup>5</sup> The available data suggest that WR 238605 is a potent blood schizonticide and may be useful in combination with chloroquine for the treatment of chloroquine-resistant *P. vivax* infections. Clinical trials to determine regimens for human prophylaxis and treatment are underway.

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1. Rieckmann KH, Davis DR, Hutton DC, 1989. *Plasmodium vivax* resistance to chloroquine? *Lancet* 2: 1183-1184.
2. Murphy GS, Basri H, Purnomo, Andersen EM, Bangs MJ, Mount DL, Ya-Ping S, Lal AA, Wiady I, Gordon J, Purwanto, Kusumo AP, Hariyanto S, Sorensen K, Hoffman SL, 1993. Chloroquine resistance in *Plasmodium vivax* malaria in Indonesia and chloroquine. *Lancet* 341: 96-100.
3. Collignon PJ, 1992. Chloroquine-resistant *Plasmodium vivax*: it may be a common problem. *Med J Aust* 157: 426.
4. Edgecomb JH, Arnold J, Young EH, Alving AS, Eichelberger L, 1950. Primaquine, SN-13272, a new curative agent in vivax malaria: a preliminary report. *J Natl Malaria Soc* 9: 285-292.
5. Pukrittayakamee S, Vanijanonta S, Chantra A, Clemens R, White NJ, 1994. Blood stage antimalarial efficacy of primaquine in *Plasmodium vivax* malaria. *J Infect Dis* 169: 932-935.
6. Cooper RD, 1994. Studies of a chloroquine-resistant strain of *Plasmodium vivax* from Papua New Guinea in *Aotus* and *Anopheles farauti* sl. *J Parasitol* 50: 789-795.
7. Cooper RD, Rieckmann RH, 1990. Efficacy of amodiaquine against a chloroquine-resistant strain of *Plasmodium vivax*. *Trans R Soc Trop Med Hyg* 54: 473.
8. Cooper RD, Milhous WK, Rieckmann KH, 1994. The efficacy of WR 238605 against the blood stages of a chloroquine-resistant strain of *Plasmodium vivax*. *Trans R Soc Trop Med Hyg* 88: 691-692.
9. Earle WC, Perez M, 1931. Enumeration of parasites in the blood of malarial patients. *J Lab Clin Med* 19: 1124-1130.
10. Peters W, Robinson BL, Milhous WK, 1993. The chemotherapy of rodent malaria: 41 studies on a new 8-aminoquinoline, WR 238605. *Ann Trop Med Parasitol* 57: 547-552.