Prophylactic Activity of a Phenanthrene Methanol (WR 33063) and a Quinoline Methanol (WR 30090) in Human Malaria

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WR 33063 (3-bromo-10-[α -hydroxy- β -(*n,n*-diheptylamino)ethyl]-phenanthrene hydrochloride) and WR 30090 (6,8-dichloro-2,3,4-dichlorophenyl-di-*n*-butylaminoethyl-4-quinolinemethanol hydrochloride) were tested for suppressive prophylactic effect on induced malaria in nonimmune volunteers living in an area where malaria is not naturally transmitted. Doses of 800 mg of WR 33063 and 690 or 460 mg of WR 30090 were given at weekly intervals to men exposed on the day of the first dose to mosquitoes heavily infected with chloroquine- and pyrimethamine-resistant strains of *Plasmodium falciparum*. WR 33063 did not interfere with early development of infection, but WR 30090 given for 8 weeks provided suppressive cures in 20 of 26 men. *P. vivax* infections similarly induced broke through WR 30090 treatment in 4 of 15 men, and most of the remainder experienced malaria after completion of the prophylactic course. No side effects of treatment were observed.

The compounds WR 33063 (3-bromo-10-[α -hydroxy- β -(*n,n*-diheptylamino)ethyl]-phenanthrene hydrochloride) and WR 30090 (6,8-dichloro-2,3,4-dichlorophenyl-di-*n*-butylaminoethyl-4-quinolinemethanol hydrochloride) were tested in nonimmune volunteers for suppressive prophylactic activity against chloroquine- and pyrimethamine-resistant *Plasmodium falciparum*. WR 30090 was also examined for its suppressive action upon *P. vivax* in man. Human tolerance to these compounds and their therapeutic effect in clinical falciparum and vivax malaria have been described (1, 5).

MATERIALS AND METHODS

Transmissions were accomplished by use of colonized *Anopheles stephensi*. Each volunteer was exposed to 10 or more heavily infected mosquitoes. The interrupted feeding method was not used, the controls being untreated volunteers exposed on each occasion to mosquitoes from the same batch as those used on the men receiving WR 33063 or WR 30090. The parasites used were the Chesson and Vietnam strains of *P. vivax* and the Philippines (Per), Malaya (Tay), and Vietnam (Smith) strains of *P. falciparum*. All three of the latter are resistant to chloroquine and pyrimethamine; the Smith strain is also resistant to chlorguanide (proguanil), and the Smith strain and to a lesser extent the Tay strain have diminished susceptibilities to quinine (2).

Absorption of WR 30090 was monitored during

the study by analysis of serum. The blood samples used were obtained from most of the participants 24 or 8 hr after supervised ingestion of the drug. Spectrophotofluorometry was undertaken on an extract containing WR 30090, the solvent being 1,2-dichloroethane added to the serum after adjustment of its pH to 4.5. Controls and blanks ensured that the test was specific and sufficiently sensitive for detection of drug concentrations as low as 0.05 μ g/ml.

The ethical guidelines of an independent University of Maryland committee were strictly observed in conducting these studies. Details of the care taken in the screening and selection of volunteers, in their daily examination after exposure to infection, and in their clinical maintenance should they develop malaria have been given elsewhere (2). Any men developing malaria received prompt treatment, and radical cure was obtained in all cases. Malaria is not transmitted naturally in Jessup, Md., where these studies were undertaken.

RESULTS

WR 33063 as a falciparum suppressive. WR 33063 in a dosage of 800 mg was administered at weekly intervals to two Caucasian volunteers, commencing on the day of their exposure to mosquitoes carrying the Smith strain of *P. falciparum*. The untreated control developed malaria in 11 days. No side effects attributable to the treatment were observed. Parasitemia appeared in volunteer JL (age, 30 years; weight, 82 kg) 13 days after exposure and in EM (age,

40 years; weight, 85 kg) 15 days after exposure, developing rapidly and requiring alternative treatment. For JL, this took the form of WR 33063, 1.6 g daily (in divided doses), and radical cure occurred.

WR 30090 (690 mg) as a falciparum suppressive. WR 30090 in doses of 690 mg was administered at weekly intervals to 18 volunteers in four groups, commencing on the day of their exposure to mosquitoes heavily infected with the Per, Tay, or Smith strain (Table 1). The untreated controls for the Per and Tay strain exposures developed malaria in 10 and 15 days, respectively. Volunteers were exposed to the Smith strain in two groups, and untreated controls developed malaria in 8 and 11 days. No side effects were attributable to the treatments, which were usually continued for 8 weeks after the infective challenge or (four men) the second of two challenges.

The method for assay of serum levels of WR 30090 was developed too late for use in the first eight cases listed in Table 1, but was used for the remainder. Levels in specimens collected 24 hr after treatment were uniformly low, ranging from 0.05 to 0.35 $\mu\text{g/ml}$, so that from the third weekly dose onwards 8-hr specimens were analyzed. These showed a wide range in WR 30090 content, from 0.00 $\mu\text{g/ml}$ on one occasion (following the fifth dose given to RF; the dose was repeated

2 days later, the only instance that this was done in the study) to 2.25 $\mu\text{g/ml}$, although all volunteers had swallowed the drugs under careful supervision and stated that they did not vomit subsequently.

Suppression and suppressive cures were obtained in 14 of the 18 men listed in Table 1. In one patient, KW, parasitemia developed after completion of the prophylactic course, and in three patients it broke through during an early stage in the course. In these three patients, parasite densities increased so rapidly that alternative treatment was required. The 24-hr drug concentrations in their sera were as follows: EH, 0.15 and 0.05 $\mu\text{g/ml}$; DJ, 0.15 and 0.23 $\mu\text{g/ml}$; and RM, 0.10 and 0.25 $\mu\text{g/ml}$.

WR 30090 (460 mg) as a falciparum suppressive. WR 30090 in doses of 460 mg was similarly administered to eight volunteers exposed to mosquitoes heavily infected with the Smith strain (Table 2). The untreated control case developed falciparum malaria after a prepatent period of 8 days. No side effects of treatment were observed.

Suppression and suppressive cures were obtained in six of the eight men. In two patients, infection broke through early in the prophylactic course and developed rapidly, making it necessary for other types of treatment to be instituted. The

TABLE 1. *Effect on P. falciparum of WR 30090 in doses of 690 mg given weekly as a suppressive prophylactic*

Volunteer ^a	Days of first and last weekly dose	Day of mosquito challenge	WR 30090 in serum ^b	Result
HC, 28, N, 67.....	0 and 49	0	—	Suppressive cure
WHu, 40, C, 93.....	0 and 49	0	—	Suppressive cure
WHa, 29, C, 73.....	0 and 49	0	—	Suppressive cure
RQ, 33, C, 62.....	0 and 49	0	—	Suppressive cure
DL, 31, C, 70.....	0 and 56	0 and 5	—	Suppressive cure
JJ, 25, C, 76.....	0 and 56	0 and 5	—	Suppressive cure
WT, 24, N, 74.....	0 and 56	0 and 5	—	Suppressive cure
KW, 27, C, 74.....	0 and 56	0 and 5	—	Parasitemia on day 78
FB, 38, N, 75.....	0 and 56	0	0.57 $\pm$ 0.21	Suppressive cure
RF, 34, C, 73.....	0 and 56	0	0.58 $\pm$ 0.40	Suppressive cure
GG, 35, N, 80.....	0 and 56	0	0.43 $\pm$ 0.24	Suppressive cure
EH, 25, C, 68.....	0 and 14	0	—	Parasitemia on day 17
DJ, 27, C, 68.....	0 and 7	0	—	Parasitemia on day 12
RM, 43, C, 93.....	0 and 21	0	—	Parasitemia on day 18
JM, 29, C, 95.....	0 and 56	0	0.72 $\pm$ 0.53	Suppressive cure
CM, 30, C, 86.....	0 and 56	0	1.25 $\pm$ 0.34	Suppressive cure
WM, 28, N, 68.....	0 and 56	0	0.89 $\pm$ 0.57	Suppressive cure
CS, 26, C, 82.....	0 and 56	0	0.85 $\pm$ 0.35	Suppressive cure

^a The initials of each volunteer are followed by his age (in years), his race (C = Caucasian; N = Negro), and his weight (in kilograms). Volunteers HC and WHu were exposed to the Per strain, WHa and RQ were exposed to the Tay strain, and the remainder were exposed to the Smith strain.

^b Concentrations of WR 30090 (in micrograms per milliliter) are the means of specimens collected 8 hr after the weekly treatments. For 24-hr levels of EH, DJ, and RM, see text.

range of 24-hr concentrations of WR 30090 in serum was 0.10 to 0.35 $\mu\text{g}/\text{ml}$; 8-hr concentrations ranged from 0.10 to 1.20 $\mu\text{g}/\text{ml}$. The initial 24-hr levels in the men who developed malaria were 0.10 and 0.15 $\mu\text{g}/\text{ml}$ for LJ and 0.10 and 0.10 $\mu\text{g}/\text{ml}$ for GS.

WR 30090 as a vivax suppressive. WR 30090 in doses of 690 and 460 mg was administered weekly to volunteers exposed as a group once to mosquitoes heavily infected with the Chesson strain or to another group exposed twice to mosquitoes with a Vietnam strain of *P. vivax*.

The untreated controls exposed on the first and second occasions to the Vietnam strain had prepatent periods of 12 and 15 days, respectively, and the Chesson control had a prepatent period of 14 days. No side effects of treatment were apparent. It should be noted that the Chesson strain challenges were made in some of the same men listed in Tables 1 and 2 as having been challenged 7 days previously with *P. falciparum*. The results of the vivax prophylactic trial are shown in Table 3 and may be summarized as follows.

TABLE 2. Effect on *P. falciparum* of WR 30090 in doses of 460 mg given weekly as a suppressive prophylactic

Volunteer ^a	Days of first and last weekly dose	WR 30090 in serum ^b	Result
CA, 24, C, 75.....	0 and 56	0.26 $\pm$ 0.15	Suppressive cure
BB, 30, N, 79.....	0 and 56	0.27 $\pm$ 0.15	Suppressive cure
FD, 31, C, 77.....	0 and 56	0.51 $\pm$ 0.16	Suppressive cure
JH, 40, C, 77.....	0 and 56	0.81 $\pm$ 0.13	Suppressive cure
LJ, 36, N, 73.....	0 and 28	0.18 $\pm$ 0.03	Parasitemia on day 28
AM, 24, C, 90.....	0 and 56	0.70 $\pm$ 0.17	Suppressive cure
JM, 28, C, 75.....	0 and 56	0.30 $\pm$ 0.04	Suppressive cure
GS, 29, C, 93.....	0 and 21	0.20 $\pm$ 0.00	Parasitemia on day 19

^a The initials of each volunteer are followed by his age (in years), his race (C = Caucasian; N = Negro), and his weight (in kilograms). All volunteers were challenged on day 0 with mosquitoes carrying the Smith strain.

^b Concentrations of WR 30090 (in micrograms per milliliter) are the means of specimens collected 8 hr after the weekly treatments. For 24-hr levels of LJ and GS, see text.

TABLE 3. Effect on *P. vivax* of WR 30090 given weekly as a suppressive prophylactic

Volunteer ^a	Dose of WR 30090 (mg)	Days of first and last weekly dose	Day of mosquito challenge	WR 30090 in serum ^b	Result	
					Day parasitemia appeared	Day follow-up ended without parasitemia
WB, 28, N, 99....	690	0 and 56	0 and 2	—	—	202
DO, 32, C, 87....		0 and 56	0 and 2	—	67	—
HT, 30, C, 70....		0 and 56	0 and 2	—	—	202
FB, 38, N, 75....		-7 and 49	0	0.57 $\pm$ 0.21	—	243
JM, 29, C, 95....		-7 and 49	0	0.72 $\pm$ 0.53	122	—
WM, 28, N, 68....		-7 and 49	0	0.89 $\pm$ 0.57	—	201
CM, 30, C, 86....		-7 and 49	0	1.25 $\pm$ 0.34	62	—
CS, 26, C, 82....		-7 and 49	0	0.85 $\pm$ 0.35	35	—
RW, 26, C, 75....		-7 and 21	0	0.85 $\pm$ 0.57	14	—
TE, 31, C, 82....	460	0 and 56	0 and 2	—	79	—
LH, 42, N, 79....		0 and 56	0 and 2	—	77	—
CA, 24, C, 75....		-7 and 49	0	0.26 $\pm$ 0.15	71	—
BB, 30, N, 79....		-7 and 49	0	0.27 $\pm$ 0.15	—	162
AM, 24, C, 90....		-7 and 49	0	0.70 $\pm$ 0.17	13	—
JM, 28, C, 75....		-7 and 49	0	0.30 $\pm$ 0.04	13	—

^a The initials of each volunteer are followed by his age (in years), his race (C = Caucasian; N = Negro), and his weight (in kilograms). Volunteers WB, DO, HT, TE, and LH were exposed to the Vietnam strain; the remainder were exposed to the Chesson strain.

^b Concentrations of WR 30090 are the means of specimens collected 8 hr after the weekly treatments. For the 24-hr levels of CS, RW, AM, and JM, see text.

Among the nine men given 690 mg of WR 30090, infection broke through the weekly treatment in two and developed after completion of the course in some others listed in Table 3. Concentrations of WR 30090 in serum with this dosage ranged from 0.10 to 0.37 $\mu\text{g/ml}$ for 24-hr specimens and from 0.10 to 2.55 $\mu\text{g/ml}$ for 8-hr specimens. The 24-hr levels in the two patients who developed early infections were as follows: CS, 0.25 and 0.25 $\mu\text{g/ml}$; and RW, 0.37 and 0.10 $\mu\text{g/ml}$.

Six men were given 460 mg of WR 30090. Breakthrough occurred in two, and parasitemia appeared upon completion of the course in some others listed in Table 3. Drug concentrations in serum ranged from 0.10 to 0.35 $\mu\text{g/ml}$ for 24-hr specimens and 0.10 to 1.20 $\mu\text{g/ml}$ for those collected after 8 hr. The 24-hr levels in the patients showing early breakthrough were 0.10 and 0.15 $\mu\text{g/ml}$ for AM and 0.23 and 0.35 $\mu\text{g/ml}$ for JM.

DISCUSSION

Although WR 33063 given to nonimmune volunteers at weekly intervals did not suppress *P. falciparum*, WR 30090 was more successful. When the latter compound was administered for 8 weeks, commencing at the time of exposure of volunteers to mosquitoes heavily infected with chloroquine- and pyrimethamine-resistant parasites, suppression and suppressive cure occurred in 14 of 18 men receiving 690 mg and in 6 of 8 men receiving 460 mg. Concentrations of WR 30090 tended to be lower in the serum of those men sustaining infection, but this was not a consistent finding. In view of the rapid diminution of WR 30090 in serum, the amount 24 hr after ingestion of 690 or 460 mg often approaching the lowest observable level, it was somewhat surprising that parasite suppression was usually achieved with weekly treatments.

The dosage of 690 mg of WR 30090, given daily for 6 days, had proved effective in the treatment of most cases of malaria caused by similar strains of *P. falciparum* (5). To determine whether the parasites breaking through our prophylactic use of the drug had developed resistance to it, the infections developing in volunteers EH (Table 1) and LJ (Table 2) were treated with the 6-day course of WR 30090. Radical cure occurred in both cases.

This protection by WR 30090 of 75 to 78% of the volunteers exposed to *P. falciparum* was

achieved without apparent side effects. It may be compared with the results obtained when we used DFD (diformyl diaminodiphenyl sulfone; WR 6798) with chloroquine or pyrimethamine under identical conditions; 93% of volunteers were protected from these strains (Clyde et al., *Mil. Med.*, *in press*).

As a prophylactic of *P. vivax*, WR 30090 was less effective. Infection broke through during the course of the weekly treatment in two of nine men receiving the 690-mg dose and in two of six receiving 460 mg, whereas the use of chloroquine alone (3, 4), or of DFD with chloroquine or pyrimethamine (Clyde et al., *in press*), ensured suppression while treatment was in progress. Upon completion of treatment with WR 30090, 6 of the 11 men who had hitherto escaped parasitemia developed it. After courses of chloroquine alone or with DFD, vivax infections are known to develop regularly, whereas this does not appear to occur after DFD with pyrimethamine (3, 4; Clyde et al., *in press*).

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