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A Phenanthrene Methanol (WR 33063) for Treatment of Acute Malaria

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WR 33063, a phenanthrene methanol, was studied in human volunteers for tolerance and toxicity. In normal volunteers, it was possible to give 4.6 g in four divided doses without adverse effect for 10 days. At this dose level, there was neither evidence of photosensitivity nor adverse renal or cardiac effect. At a dose level of 1.6 g in four divided doses for 6 days, WR 33063 cured 18 of 23 nonimmune volunteers infected with the Smith strain of *Plasmodium falciparum* from Vietnam. In addition, infections due to the Marks and Braithwaite Vietnam strains were also treated because these strains represent a major therapeutic challenge to chloroquine; six of six and two of three volunteers, respectively, were cured. With the Malayan Camp strain, 1.6 g in four divided doses for 6 days cured all of five volunteers. The African Uganda I strain of chloroquine-responsive malaria was even more responsive to WR 33063; all of six men who received 1.6 g in four divided doses for 6 days were cured, and all of three men who received this same dosage for 3 days were cured. One subject infected with a Haitian strain of *P. falciparum* was treated and cured. Blood-induced infections with the Chesson strain of *P. vivax* also responded well to WR 33063 with four of five men cured. In all, 52 men received WR 33063 in tolerance trials, and 59 men with experimental malaria and one man with clinical malaria were treated with WR 33063.

Several phenanthrene methanols received clinical trials in human malaria during World War II. Coatney et al. (1) reported their experience in the published literature, and others reported their experience with this family of compounds in unpublished malaria reports (A. S. Alving and L. Eichelberger, Unpublished Malaria Report 723, 31 August 1946; J. A. Shannon, Unpublished Malaria Report 224, 18 September 1944). Four phenanthrene methanols were studied in man during the wartime period. Because interest during World War II was focused on the tissue forms of vivax malaria, little information was sought on the response of *Plasmodium falciparum* to members of this drug family. It was established, however, that this group of compounds possessed good blood schizontocidal action in vivo against *P. vivax*.

WR 33063 (6-bromo-α-[diheptylaminomethyl]-9-phenanthrenemethanol, Fig. 1) was first synthesized by May and Mosettig (6) during the World War II studies, but it did not receive a clinical trial.

One problem recognized early in the use of the phenanthrene methanols was that of induced phototoxicity. Unless a phenanthrene methanol could be found which did not induce phototoxicity, the recognized antimalarial activity could not be exploited in man. Renewed interest developed in WR 33063 because (i) it was well tolerated, as shown by the fact that the acute oral LD_{50} in mice, rats, and guinea pigs was greater than 10,000 mg/kg of body weight, and that 14-day oral administration of 250 mg/kg in rhesus monkeys showed no toxicity; (ii) it was relatively free from phototoxicity in animal studies (7); and (iii) it showed good performance in screening tests of antimalarial drugs in animals. In addition to the concern about phototoxicity, there was also concern about gastrointestinal absorption derived from the experience of Alving and Eichelberger (*unpublished data*) with SN 8867 (α-[dinonylaminomethyl] - 9 - phenanthrenemethanol) and about cardiac and renal effects from studies of another related drug, SN 1796 (α-[diamylaminomethyl]-1,2,3,4-tetrahydro-9-phenanthrenemethanol), which produced sinus bradycardia, dysuria, and cylindruria in man.

Because of early observations on related compounds, the first human exposure to WR 33063

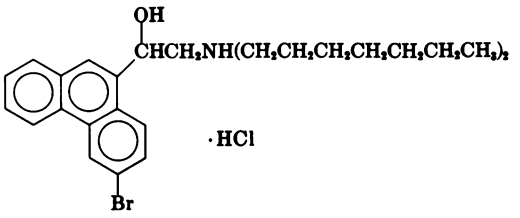

FIG. 1. *Structural formula of WR 33063, 6-bromo-α-(di-n-heptylaminomethyl)-9-phenanthrene methanol-hydrochloride.*

was carried out with special attention directed at potential sources of toxicity. After determining the human tolerance and toxicity of WR 33063, the therapeutic effectiveness of the compound was examined in several dosage schedules against malaria in a panel of humans. This panel was designed to represent the range of therapeutic challenges presented by malaria around the world at the time of this study.

The chemical synthesis, drug formulation, animal therapeutic screening, animal toxicology, and pharamcology were carried out as part of the U.S. Army Malaria Research Program. These data were compiled and the drug was approved for human use by the Office of the Surgeon General and by the U.S. Food and Drug Administration.

MATERIALS AND METHODS

The study divides into two phases. Phase I involved an appraisal of human tolerance and toxicity. In this phase, 52 men volunteered, none of whom had had malaria. In phase II, the therapeutic efficacy of WR 33063 was appraised in volunteers with induced malaria. The ethical aspects of these studies were supervised by independent institutional review committees.

A careful medical history and physical examination for all volunteers preceded admission to the malaria research units. In addition, a careful explanation of the character and risks of WR 33063 and malaria was presented orally and in writing to each volunteer; all volunteers gave an informed consent. Routine medical laboratory work included the following determinations: hematocrit value, total white blood cell (WBC) count, differential white cell count, platelet count, blood glucose, blood urea nitrogen (BUN), alkaline phosphatase, serum glutamic oxalacetic transaminase levels (SGOT), and urinalysis. Additional studies at admission included chest X rays, serology, blood typing, tuberculin skin test, and Australia antigen determinations.

Phase I: tolerance and toxicity studies. The tolerance and toxicity studies were based on a graded increase in dosage of drug as shown in Table 1. The subjects (three on drug and three on placebo) were interviewed daily for evidence of subjective intolerance. The following laboratory tests were performed every 1 to 3 days, depending on the duration of drug administration: hematocrit, total and differential WBC count, platelet count, blood glucose, BUN, alkaline phosphatase, SGOT, and urinalysis. Electrocardiograms were obtained before and 4 hr after drug exposure. In addition, four subjects were monitored by a continuously recording electrocardiograph for an 8-hr period after drug administration. This included one man who received a single dose of 4.6 g of WR 33063.

TABLE 1. *Phase I studies with WR 33063 in normal subjects*

No. of subjects	Total daily dose (g)	Divided dose (times/day)	No. of days
3	0.05	1	3
3	0.1	1	3
3	0.2	1	3
3	0.4	1	3
3	0.8	1	3
3	1.6	1	3
3	3.1	1	3
3	4.6	1	3
6	1.6	4	6
10	1.6	4	10
3	2.4	4	10
3	3.2	4	10
3	4.0	4	10
3	4.6	4	10

Evidence of phototoxicity was appraised by the method of Ison and Blank (4). Prior to receiving WR 33063, the volunteer was exposed to measured amounts of direct ultraviolet (UV) light from a Hanovia light to determine the minimal erythematous dose (MED). Daily during drug therapy, 20 and 60 MED of UV light was filtered through ordinary plate glass, and the skin was examined 2 and 24 hr after exposure for evidence of erythema.

Phase II: therapeutic efficacy. The volunteers were usually infected by intravenous inoculation of 10^6 to 10^7 parasitized red blood cells (RBC). The parasitized RBC were preserved in a blood-citrate-glycerol preparation that was frozen in liquid nitrogen and thawed just prior to injection. A few volunteers were infected with colonized *Anopheles stephensi*.

A total of 59 volunteers with induced malaria were studied, and one man was treated for clinical malaria. They were all observed in hospital wards with 24-hr nursing and medical coverage. Daily parasite counts were done before and during the initial parasitemia. Routine laboratory tests previously detailed were also performed at frequent intervals. Treatment with WR 33063 was usually begun after 1 day of fever and after 2 or more days of confirmed parasitemia. Examination

of thick smears (3) was done three times a week during the 60-day post-therapy observation period for evidence of recrudescence.

The therapeutic response to infections with both *P. vivax* and *P. falciparum* was studied. Five volunteers were infected with the Chesson strain of *P. vivax*. This strain has been extensively studied by many investigators using a variety of drugs so that it provides an important basis of comparison.

Seven different strains of *P. falciparum* with various degrees of drug resistance were used. Their anticipated response to three standard antimalarial drugs is shown in Table 2. Nine men were infected with the Uganda I strain of *P. falciparum* which was obtained from a child in Kampala, Uganda. This strain is susceptible to all of the commonly used antimalarial drugs (5). One man was infected with a Haitian strain of *P. falciparum* which is also quite susceptible to chloroquine.

Eight volunteers were infected with the Malayan Camp strain of *P. falciparum* (2). This strain is moderately resistant to chloroquine and severely resistant to pyrimethamine, but it is usually susceptible to high doses of quinine.

Six volunteers were infected with the Vietnam Marks strain of *P. falciparum*, which was obtained from a soldier from Vietnam who had had multiple falciparum recrudescences after treatment with quinine, pyrimethamine, dapsone, and chloroquine. He was ultimately cured with WR 33063 (C. J. Canfield et al., *unpublished data*). Twenty-seven men were treated with the Vietnam Smith strain of *P. falciparum*. The Smith strain has been unresponsive to chloroquine in acceptable doses and to antifolic acid compounds, and it has a relative resistance to quinine, sulfonamide, and sulfones. These latter compounds are usually required in high doses and in combinations with pyrimethamine to effect cures. Three men were infected with the Braithwaite strain of *P. falciparum* from Vietnam which is resistant to chloroquine and quinine. The Crocker strain of *P. falciparum* was obtained from a young man recently returned from Vietnam who had been unsuccessfully treated for malaria at a military hospital. The Crocker strain has been shown to be resistant to chloroquine, quinine, sulfonamides, and folic acid antagonists.

TABLE 2. *Response[a] of various strains of P. falciparum malaria to standard antimalarial chemotherapy[b]*

Strain	Chloroquine	Pyrimethamine	Quinine
African			
Uganda I	S	S	S
Caribbean			
Haitian	S	S	S
Malayan			
Camp	R I–R II	R II	S
Vietnam			
Marks	R II	R III	R I
Smith	R III	R III	S–R III
Braithwaite	R II	S	R I
Crocker	R I	?	?

[a] Response: S, susceptible, radical cure; R I, resistant, asexual parasitemia is cleared but recrudesces; R II, resistant, parasite density is reduced but not cleared; R III, resistant, parasitemia not affected.

[b] The results were obtained with the following dosage schedules for the three drugs (all expressed as grams of base): chloroquine, 1.5 g over 3 days; pyrimethamine, 0.15 g over 3 days; quinine, 11.3 g over 7 days.

The location and conditions of these studies precluded the possibility of malarial infections other than those that were experimentally induced. Any reappearance of falciparum asexual parasites in the peripheral blood after therapy must then be due to an incomplete blood-schizontocidal effect of the drug.

RESULTS

Phase I: tolerance and toxicity. With the treatment regimens tested (Table 1), there were no subjective complaints that could be related to WR 33063 except for two patients receiving 3.2 g in four divided doses who experienced mild nausea which disappeared after 2 days.

There were no significant changes in the hematocrits, total WBC counts, differential WBC counts, or platelet counts attributable to the drug. There were no consistent changes in the results of the serum chemistry tests. Some patients had minor and transient elevations of SGOT. However, the volunteer population has had a higher than normal incidence of occult hepatitis, and transient elevations of the serum enzymes were observed during control periods. The observed changes during drug administration were not dose-related and were never greater than 3 standard deviations from normal.

Examination of the urinary sediments showed some specimens with cells and casts. In the volunteer population, there was a fairly high level of lower urinary tract infection which accounts for some degree of abnormality in the sediment. However, there were no changes in the character of the sediment during treatment except for one patient who developed gross hematuria while receiving WR 33063 at 1.6 g in divided doses for 6 days. He had had a substantial level of microscopic hematuria prior to administration of the drug (15 to 25 RBC per high-power field). Hematuria diminished sharply on the second day of

therapy, and by the sixth day of therapy there were only 0 to 2 red cells per high-power field. It is believed that the hematuria was unrelated to WR 33063.

The electrocardiograms and 8-hr Holter monitor recordings obtained after drug administration were scanned for evidence of abnormality in electrical activity. This survey failed to show any evidence of cardiac toxicity at any dose.

In a few instances, the results of the study of phototoxicity had to be discounted because of a technical fault in the procedures. This reduced the total number of valid studies of phototoxicity to somewhat less than the total number of men who received WR 33063. In none of the 92 volunteers who were studied was there evidence of phototoxicity. Under the conditions of the study, the control period for erythema from unfiltered light averaged 20 sec. The average period of exposure to filtered light for challenge with drug was 20 min.

Phase II: therapeutic. The first clinical trials represented a dose-searching effort with small doses over a 1- to 3-day period. The earliest studies were carried out in volunteers who had had a recrudescence after treatment with another drug or who were reinfected, partially immune volunteers. This lessened the risk and provided information on probable effective dose levels. After the preliminary study in four men (not included in Table 3), 15 nonimmune volunteers infected with various strains were treated with a less than 6-day regimen of WR 33063 (Table 3).

The Smith strain was not responsive to any dose employed for 3 days or less, whereas the Camp strain could be cured at doses of 1.6 g given in four divided doses for 3 days. This seemed to give a clear division of drug responsiveness among the Uganda I, Camp, and Smith strains, with the Smith strain measurably less susceptible to therapy with WR 33063.

Consequently, the therapy was extended to 6 days with 1.6 g daily in divided doses. Parasitemia cleared and patients became afebrile with the Uganda I strain within 4 days from the start of treatment, with the Camp strain within 8 days, with the Marks strain within 5 days, and with the Smith strain within 4 days. The 6-day course readily cured infections caused by Uganda I, Camp, and Marks strains of *P. falciparum*. It also gave a very gratifying rate of cure for the Smith strain; 18 of 22 subjects were cured, and 1 subject required a second course of therapy. There were three other recrudescences with the Smith strain and one subject who did not clear parasitemia. Infected blood from the subject who did not clear parasites was subinoculated into two other volunteers. One of these volunteers was cured and one cleared parasitemia but underwent recrudescence. One subject infected with the Vietnamese Braithwaite strain and the patient Crocker were not cured.

Except for the single volunteer who did not have parasite clearance with a 6-day course of WR 33063, clinical responses and parasite suppression were rapid and there was no clinical or laboratory evidence of adverse drug effect.

In Table 4, a summary is given of the schizontocidal effect of WR 33063 against blood-induced *P. vivax*. The administration of 1.6 g in divided doses for 3 days cured one of two volunteers and 1.6 g in divided doses for 6 days cured all of three volunteers.

Very few gametocytes were observed after therapy with WR 33063 in either falciparum or vivax infections. Four volunteers were exposed to *A. stephensi* mosquitoes without a successful mosquito infection.

DISCUSSION

The phenanthrene methanol WR 33063 clearly extends the range of therapy of drug-resistant *P. falciparum* malaria. On the basis of results from the several strains of *P. falciparum* tested, WR 33063 may have the broadest spectrum of the synthetic drugs tested in volunteers for clinical cure of *P. falciparum*. This comparison includes chloroquine alone, sulfalene alone, sulfalene plus trimethoprim, sulfones plus trimethoprim, sulfa plus pyrimethamine, and other less important drugs and combinations. It does not include a comparison with the Army program of chloroquine, quinine, pyrimethamine, and sulfone, because we do not have sufficient data to make a close comparison. Not only is the therapeutic spectrum of WR 33063 broad, but the clinical response to therapy is rapid and the adverse effects of the drug have been nonexistent in all volunteers.

Despite this very great improvement in action, WR 33063 has some shortcomings. It appears that some *P. falciparum* strains have a relative resistance to the doses used. These strains, as well as the phenomenon of induced resistance, are under further study. The drug works best in divided daily doses, and a minimum of 3 days of therapy seems to be required for most responsive strains. Resistant strains, such as the Smith strain, require 6 days or more of therapy.

Although our data are not conclusive on this point, it seems probable that the susceptibility of malaria strains to WR 33063 is correlated with their susceptibility to chloroquine. Thus, the Uganda and Haiti strains, which are most susceptible to chloroquine, are also most suscep-

TABLE 3. *Treatment of acute P. falciparum malaria with WR 33063*

Malaria strain and patient[a]	Total daily dose (g)	Divided doses (times/day)	No. of days	Parasite count	Days to recrudescence	Cure (days of follow-up)
African Uganda I						
Hol(J)	1.0	1	3	8,640	—	25
Jon(J)	1.6	4	3	2,880	12	—
And(J)	1.6	4	3	11,270	—	54
Val(J)	1.6	4	6	3,070	—	74
Lam(J)	1.6	4	6	6,040	—	83
Col(J)	1.6	4	6	7,810	—	60
Bro(J)	1.6	4	6	8,120	—	60
Dor(J)	1.6	4	6	1,500	—	64
Mad(J)	1.6	4	6	1,130	—	61
Caribbean Haiti						
Mor(J)	1.6	4	6	300	—	60
Malayan Camp						
Gib(J)	1.6	4	3	64,870	—	88
Boo(J)	1.6	4	3	970	—	66
Cou(J)	1.6	4	3	1,300	—	113
How(J)	1.6	4	6	12,460	—	60
Sch(J)	1.6	4	6	22,300	—	84
Fos(J)	1.6	4	6	480	—	60
Lan(J)	1.6	4	6	720	—	60
Car(J)	1.6	4	6	600	—	63
Vietnam Smith						
Har(J)	0.8	1	3	80	12	—
	1.8	1	3	1,200	15	—
Str(J)	0.8	1	3	510	24	—
	1.0	1	3	450	14	—
Lac(J)	3.2	1	1	3,780	2	—
	1.0	1	5	1,520	16	—
Old(J)	1.2	4	3	2,540	18	—
Sow(J)	1.6	4	3	3,810	10	—
Phi(J)	1.6	4	6	1,120	—	60
Pat(J)	1.6	4	6	5,690	—	79
Hop(J)	1.6	4	6	21,540	—	64
Cri(J)	1.6	4	6	130	—	61
Kno(J)	1.6	4	6	8,020	NC[b]	—
Slu(J)	1.6	4	6	120	—	61
Tay(J)	1.6	4	6	1,440	—	74
Tyl(J)	1.6	4	6	330	—	69
Huf(J)	1.6	4	6	5,840	24	—
	1.6	4	6	140	—	60
Mad(J)	1.6	4	6	380	—	60
Dea(J)	1.6	4	6	290	—	60
Hal(J)	1.6	4	6	6,200	32	—
Pat(J)	1.6	4	6	1,470	—	72
Lon(J)	1.6	4	6	60	—	60
Her(J)	1.6	4	6	50	—	60
Smi(J)	1.6	4	6	1,740	—	65
Dea(M)	1.6	4	6	1,540	—	60
Osi(M)	1.6	4	6	11,580	27	—
Lor(M)	1.6	4	6	16,020	—	60
Kol(M)	1.6	4	6	15,900	—	60
Mar(M)	1.6	4	6	3,280	—	60
Hod(M)	1.6	4	6	6,840	41	—
Vietnam Braithwaite						
Bra(M)	1.6	4	6	2,840	—	66
Coc(M)	1.6	4	6	3,240	21	—
	1.6	4	6	270	19	—
Dow(M)	1.6	4	6	7,440	—	66

TABLE 3—Continued

Malaria strain and patient[a]	Total daily dose (g)	Divided doses (times/day)	No. of days	Parasite count	Days to recrudescence	Cure (days of follow-up)
Vietnam Marks						
Ser(S)	1.6	4	3	20	—	60
Pon(S)	1.6	4	6	30	—	60
Was(S)	1.6	4	6	50	—	60
Jac(S)	1.6	4	6	160	—	60
Hou(S)	1.6	4	6	410	—	60
Mez(S)	1.6	4	6	310	—	60
Vietnam Crocker						
Cro(J)	1.6	4	6	55,820	39	—

[a] The letter in parentheses following the shortened form of the patient's name represents the institution where the test was performed.
[b] No parasite clearance.

TABLE 4. *Treatment of acute P. vivax malaria (South Pacific, Chesson strain) with WR 33063*

Patient	Total daily dose (g)	Divided doses (times/day)	No. of days	Parasite count	Cure (days of follow-up)
Car(J)	1.8	1	3	90	64
Rog(J)	1.6	4	3	6,600	—[a]
Wya(J)	1.6	4	6	910	62
Wil(J)	1.6	4	6	860	123
Hen(J)	1.6	4	6	16,250	61

[a] Recrudescence at 31 days.

tible to WR 33063. The Camp strain, with an intermediate resistance to chloroquine, is quite susceptible to WR 33063. The Smith strain, which is one of the most chloroquine-resistant strains, is also most resistant to WR 33063.

An inquiry into the ease of inducing resistance is not yet complete, but treatment failure at the largest doses used in therapy was seen and will probably occur again. Studies are underway to examine the relationship of drug levels to the incidence of therapeutic failure. In addition to a very considerable trophozoite effect, WR 33063 either prevents gametocyte formation by its rapid trophozoite action or is gametocidal.

Blood-induced *P. vivax* infections were satisfactorily cured (four of five) with WR 33063. Although it is not anticipated that this drug will be competitive with chloroquine, these results suggest that mixed infections due to *P. falciparum* and *P. vivax* can be satisfactorily treated with WR 33063 for termination of acute attacks and elimination of erythrocytic stages of both plasmodia.

Some of the early promise of the phenanthrene methanols as therapeutic agents was tempered by the concurrent appearance of severe phototoxicity in animals and a few men. Results of standardized phototoxicity testing indicate that this undesirable characteristic of the family of compounds has been avoided in WR 33063. This confirms the predictions of Rothe and Jacobus (7), who were able to sort out WR 33063 from the groups of sensitizing phenanthrene compounds. It remains, however, to be seen what exposure to severe sunlight in a large number of subjects will do. A certain proportion of the phototoxicity of other drugs is uncovered only in this way.

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