Comparative Study of WR 225448 and Primaquine in the *Plasmodium cynomolgi* - Rhesus Monkey Radical Curative Model

Principal Investigators : John L. Brown, LTC, VC
Frank E. Chapple, III, MAJ, VC
Richard G. Andre, MAJ, MSC
Prayot Tanticharoenyos, DVM
Kwanyuen Lawhaswasdi, DVM

Associate Investigators : David E. Davidson, LTC, DVM
Markpol Tingpalapong, DVM, LL. B.
Paul K. Hildebrandt, COL, VC

OBJECTIVES :

1. To compare the efficacy of WR 225448-chloroquine with the radical curative combination of primaquine-chloroquine.

2. To compare the efficacy of WR 225448 alone with the combination WR 225448-chloroquine.

3. To determine whether WR 225448 is efficacious in a single oral dose.

BACKGROUND : In rhesus monkeys infected with sporozoites of *P. cynomolgi*, primaquine (in combination with chloroquine) is a radical curative drug. In this combination, primaquine cures most animals at a total dose of 3.5 mg base per kg body weight, whether that dose is a single dose or divided into 3 or 7 daily doses (1).

In man, the toxicity of primaquine precludes administration in a single curative dose. Thus, to achieve a radical cure of *P. vivax* in man, the dose is ordinarily given in divided doses over 14 to 21 days (in conjunction with a 3-day course of chloroquine).

WR 225448, an experimental 8-aminoquinoline, appeared to be as potent as primaquine as a tissue schizonticide and had exceptionally potent blood schizonticidal activity. The toxicity of WR 225448 was not investigated, but preliminary evidence suggested that it was at least no more toxic than primaquine.

Because of the apparent blood schizonticidal activity of WR 225448, the ability to achieve a radical cure of sporozoite induced *P. cynomolgi* without simultaneous administration of a second drug such as chloroquine was considered a real possibility. Since preliminary testing of WR 225448 indicated that it had a better therapeutic index than primaquine, it was decided to also test its efficacy by a single oral dose.

The dose ranges (Table 1) selected for this study were based on assumptions that the new lot of WR 225448 used in this study would have approximately the

potency as the succinate salt used in preliminary studies and that WR 225448 would cure at the same total dose whether it was given in a single dose or in seven divided doses.

METHODS : Rhesus monkeys were inoculated intravenously with 5-20 X 10^5 *P. cynomolgi* sporozoites produced in *Anopheles balabacensis* mosquitoes. Each monkey was then assigned, by random selection, to a particular drug-dose regimen.

Administration of drug in each monkey was initiated on the day after its initial parasitemia reached 5000/cmm (chloroquine was given beginning on this day in the appropriate groups). Parasitemia was determined in each monkey, by blood smears, three times prior to inoculation, daily from day six post-inoculation until three days after parasitemia is suppressed to zero, then every other day through day 40 and twice weekly thereafter. During relapses or recrudescences of parasitemia, counts were made daily.

Monkeys in which parasitemia were cleared by drug were monitored thru day 40 at which time they were splenectomized and then monitored for an additional 40 days. If negative at the end of this period they were considered cured. Monkeys in which parasitemia was not cleared by drug were terminated on day 40.

Monkeys in which the parasitemia was cleared by drug but then reappeared before day 40 were treated with chloroquine phosphate, orally, 5 mg/kg for seven days (whether or not chloroquine was included in the original regimen). If the parasitemia was cleared by the chloroquine, splenectomy was performed 20 days after clearance and the monkey monitored an additional 30 days. Splenectomized monkeys in which parasitemia reappeared were treated with chloroquine phosphate, orally, 5 mg/kg for 7 days. Those animals in which the parasitemia was cleared by this chloroquine treatment were monitored for an additional 50 days.

RESULTS : Results for each treatment regimen are summarized in Table 2. Results of each drug regimen were categorized as Cure, Relapse, or Recrudescence based on the following definitions:

1. Cure : No reoccurrence of parasitemia following original treatment.

2. Relapse : Failure of the curative drug to clear all tissue parasites. Confirmed by administration of chloroquine to all monkeys with recurrent parasitemia followed by a <u>temporary</u> clearance of the parasitemia.

3. Recrudescence : Failure of the curative drug to clear all blood parasites. Confirmed by administration of chloroquine to all monkeys with recurrent parasitemia followed by a <u>permanent</u> clearance of parasitemia.

A combination of WR 225448 and chloroquine was clearly the most potent regimen. In combination with chloroquine, the minimum curative dose of WR 225448 was 0.876 mg base per kg body weight. Administered singly WR 225448 was not curative at the highest dose utilized, 1.75 mg base per kg body wt. Results indicate that WR 225448 is equally effective as a tissue schizonticide when administered either single or in combination with chloroquine.

Primaquine was not consistantly curative at any regimen tested. The lowest

dose of primaquine which produced a cure was 3.5 mg/kg body wt. Therefore, using base weight of the drug as the criteria for evaluation, WR 225448 is 4-8 times as potent as primaquine as a tissue schizonticide when administered as a single oral dose. Both primaquine and WR 225448 were weighed and administered in a similar random manner. One can only speculate in regard to why primaquine in combination with chloroquine was not consistantly curative at 3.5 and 7.0 mg base per kg body weight.

Although WR 225448 is more potent as a tissue schizonticide, it does possess blood schizonticidal activity. Previous studies have indicated that WR 225448 is an effective blood schizonticide at 5.5 mg base per kg body weight, but not at 1.75 mg base per kg body wt. Results of this study are corroborative. The blood schizonticidal MCD_{50} apparently lies between 5.5 and .175 mg per kg. A second study using other dose regimens is now being formulated.

REFERENCE :

1. Brown, J.L., et al., AFRIMS Annual Progress Report 1976-77.

Table 1. Treatment Regimens

Dosage of WR 225448 or Primaquine mg Base/Kg body weight	TREATMENT GROUPS							Dosage of Controls mg Base/Kg Body weight
	WR225448*	WR225448* +Chloroquine**	Primaquine* +Chloroquine**	CONTROLS				
				Primaquine*	Chloroquine**	Vehicle**		
7.0			2					
3.5			2					
1.75	2***	2	2					
0.875	2	2						
0.4375	2	2						
				2			7.0	
					2		3.1	
						2	0	

1. In all cases when Chloroquine was given in conjunction with another drug (WR 225448 or Primaquine) the dose of Chloroquine was 3.1 mg Base/Kg Body weight.

2. * Single oral dose
   ** Seven daily oral doses
   *** Number of Monkeys/dose

Table 2. Summary of Results

Dosage of WR 225448 or Primaquine mg Base/Kg Body weight	TREATMENT GROUPS						Dosage of Controls mg Base/Kg Body weight
				CONTROLS			
	WR225448*	WR225448* +Chloroquine**	Primaquine* +Chloroquine**	Primaquine*	Chloroquine**	Vehicle**	
7.0			G286-Recrudescence G308-Relapse				
3.5			G278-Cure G294-Relapse				
1.75	G335-Recrudescence G277-Recrudescence	G267-Cure G307-Cure	G228-Relapse G309-Relapse				
0.875	G273-Recrudescence G261-Recrudescence	G260-Cure G283-Cure					
0.4375	G279-Recrudescence G293-Recrudescence	G272-Relapse G274-Relapse					
				G264-Recrudescence G296-Recrudescence			7.0
					G246-Relapse G262-Relapse		3.1
						G291-No Effect G253-No Effect	0

* - Single oral dose
** - Seven daily oral doses
*** - Monkey number/result