

Evaluation of Experimental Antimalarial  
Drugs in Rhesus Monkeys Infected with *Plasmodium cynomolgi*

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**OBJECTIVE:** To evaluate the effectiveness of selected experimental drugs against *Plasmodium cynomolgi* malaria in rhesus monkeys. Results of these studies in subhuman primates are used in the U.S. Army antimalarial drug development program to guide the design of further animal experiments and to aid in the selection of drugs for human trials.

**DESCRIPTION:** These are a continuation of studies initiated in 1971, and reported with details of methodology in the SEATO Medical Research Laboratory Annual Reports for 1972-1974. These studies include an evaluation of blood schizonticidal activity of candidate compounds in rhesus monkeys inoculated intravenously with  $5 \times 10^8$  parasitized erythrocytes obtained from donor monkeys infected with *Plasmodium cynomolgi* strain B. Test drugs are administered daily by stomach tube for seven days beginning four days after the parasite inoculation. Suppression of parasitemia is indicative of blood schizonticidal activity, and monkeys in which parasitemia fails to reappear for one month after splenectomy at 30 days are considered cured. Drug tolerance studies, in which a minimum tolerated dose is established and major toxic effects characterized, are also conducted in rhesus monkeys.

This year pilot studies to establish a rhesus monkey test system to evaluate candidate compounds for causal prophylactic or radical curative activity have been initiated, using methodology patterned after that developed in other laboratories. Techniques for infecting *Anopheles balabacensis* with *Plasmodium cynomolgi* strain B and for reproducibly infecting rhesus monkeys with infective sporozoites are being refined.

**RESULTS:**

**Blood Schizonticidal Tests:** This year 19 experimental antimalarial drugs were evaluated for blood schizonticidal activity. Minimum curative doses are indicated in Table 1. A number of the newer 8-aminoquinolines, particularly WR 182232 have greater activity against blood schizonts and are less toxic than primaquine. Two novel compounds, WR 194965 and WR 204165 have excellent antimalarial activity. A study performed with a formulated mixture of sulfadiazine and WR 158122 (a 2,4-diaminoquinazoline) suggests that these compounds are synergistic in combination. Minimum curative doses for the individual components of the mixture were 100 and 1.0 mg/kg respectively.

**Tolerance Tests:** Drug toxicity studies were conducted with six compounds. Results are summarized in Table 2. Hepatic toxicity has been encountered in two 8-aminoquinolines (WR 181023 and WR 182232), and in two benzamidines (WR 4931 and WR 199385).

**Sporozoite Induced Tests:** The suitability of *Anopheles balabacensis* for mass production of infective *P. cynomolgi* strain B sporozoites has been established. In a series of preliminary experiments, this mosquito has been demonstrated to be hardy and an aggressive feeder on rhesus monkeys. Engorgement rates have regularly been above 85%, and 90% or more of the engorged mosquitoes have developed moderate to heavy sporozoite infections. Baseline studies with primaquine are in progress, and the testing of experimental compounds in prophylactic and radical curative regimens in the sporozoite-induced system will be initiated in the coming year.

SUMMARY: Six antimalarial compounds have been evaluated for toxicity in rhesus monkeys, and 19 for blood schizonticidal activity against *P. cynomolgi* strain B. Sporozoite-induced test systems are being developed to permit the evaluation of causal prophylactic and radical curative activity of antimalarial drugs in rhesus monkeys.

Table 1. Summary of Blood Schizonticidal Tests in Rhesus Monkeys

Type of Compound	WRAIR Drug Number	Minimum Curative Dose (mg/kg/day)
4-Aminoquinoline	1544	10.0
8-Aminoquinoline	2975 (Primaquine) 6020 181023 (lot 1) 181023 (lot 2) 182232 182234	NC <sup>1</sup> (31.6) NC <sup>1</sup> (100.0) 10.0 31.6 3.16 10.0
4-Quinolinemethanol	184806	10.0
9-Phenanthrene-methanol	181018	31.6
4-Pyridinemethanol	182231	10.0
Sulfonamide	4629 (Sulfalene) 4873	100.0 NC <sup>1</sup> (100.0)
Miscellaneous	5473 5949 (Trimethoprim) 25979 (Nitroguanil Hydrochloride) 190830 194965 204165	3.16 100.0 31.6 100.0 3.16 3.16
Combination Study	7557 (Sulfadiazine) } 10:1 158122	1.0:0.1

<sup>1</sup> Not curative. The compound had suppressive activity, but did not cure at the maximum dose tested. The maximum tested dose is indicated in parentheses.

Table 2. Summary of Drug Tolerance Studies in Rhesus Monkeys

Compound Number	Maximum Tolerated Dose (mg/kg/day)	Principle Toxic Effect
WR 4931	< 3.16 (I.M.)	Liver Damage
WR 172435	< 316 (Oral)	Emesis
WR 181023	< 10 (Oral)	Liver Damage
WR 182232	< 31.6 (Oral)	Liver Damage
WR 184806	10 (Oral)	Emesis
WR 199385	< 3.16 (I.M.)	Liver Damage