

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 *P. cynomolgi* malaria in rhesus monkeys. These studies are coordinated by the Division of Medicinal Chemistry, Walter Reed Army Institute of Research, and the results are used to aid in selection of more effective antimalarial drugs for human use.

BACKGROUND : This is a continuation of studies initiated in 1971. A chronological report of methodology and results are available in SEATO Medical Research Laboratory Annual Reports for 1971 through 1975. During 1975-76 work was oriented toward perfection of a sporozoite induced test system for evaluation of radical curative drugs.

DESCRIPTION :

Blood Schizonticidal Tests : Experimental drugs are evaluated in rhesus monkeys (*Macaca mulatta*), which are infected by intravenous administration of 5×10^8 parasitized erythrocytes obtained from donor monkeys infected with *P. cynomolgi* strain B. Post-inoculation day 4, test drugs are administered orally (by gastric intubation) for seven days. Suppression of parasitemia is indicative of blood schizonticidal activity, and post-treatment day 20, splenectomy is performed on monkeys in which there is no evidence of parasitemia. Splenectomized monkeys which are continuously negative for malarial parasites through post-treatment day 50 are considered cured.

Sporozoite Induced Radical Curative Tests : *A. balabacensis* mosquitoes were used for production of sporozoites. A rhesus monkey, inoculated with *P. cynomolgi* strain B approximately one month prior to the anticipated mosquito feed, was used as a donor. Splenectomy was performed to assure a rising parasitemia at the time of mosquito feed. Once the parasitemia reached 10^4 parasitized erythrocytes per cmm, but prior to the period of descending parasitemia, three separate batches of mosquitoes were fed on consecutive days.

Post-feed day 6, mosquitoes were examined for gut oocysts. Twenty to 80 oocysts per gut were considered optimum for sporozoite development. Absence of oocysts was indicative of an unsuccessful mosquito feed. Post-feed day 14, sporozoites were harvested and diluted to contain $5-20 \times 10^5$ sporozoites per ml, (approximately 15 mosquito salivary gland pairs per ml.). Each test monkey was inoculated intravenously with one ml of the sporozoite suspension.

Parasitemia normally developed in eight days, and once there were $5-25 \times 10^3$ parasites per cmm, drugs were administered orally for seven days. Chloroquine phosphate (WR 1544) at 4.0 mg/kg/day was given simultaneously with all test drugs by the same route. Blood was examined for malarial parasites daily for 12 days, then every two days thereafter. Monkeys which remain negative through post-treatment day 20 were splenectomized and observed for an additional 33 days. If parasites were absent through post-treatment day 53 the drug was considered curative.

RESULTS :

Blood Schizonticidal Tests : A total of 12 experimental drugs were evaluated for blood schizonticidal activity. Minimum curative doses are indicated in Table 1. Combination studies were conducted to meet special drug development requirements. Four sets of drug combinations were tested and the initial results shown in Table 2 suggest that there is synergistic action.

Sporozoite Induced Radical Curative Tests : Development of sporozoites following engorgement of mosquitoes on splenectomized donor monkeys was not consistent. In order to obtain a more reliable system for mass sporozoite production, plans were initiated to utilize intact donor monkeys for future experiments. A total of eight experimental drugs were evaluated for radical curative properties. A minimum curative dose for each is indicated in Table 3.

SUMMARY : Rhesus monkeys infected with trophozoite induced *P. cynomolgi* strain B were used to evaluate twelve single drugs and four drug combinations for blood schizonticidal activity. A sporozoite induced test system was used to evaluate eight drugs for radical curative activity.

Table 1. Summary of Blood Schizonticidal Tests in Rhesus Monkeys

Type of Compound	WRAIR Drug Number	Minimum Curative Dose (mg/kg/day)
Pyridine methanol	180409 AC	10.0
	180009 AD	10.0
	180117	10.0
Phenanthrene methanol	33063	* NC (31.6)
2,4-Diamino quinazoline	150012	3.16
Quinoline methanols	142490	31.6
	199426	10.0
Miscellaneous	151136	* NC (100.0)
	38839	3.16
	194905	* NC (31.6)
	99210	31.6
	6171	* NC (31.6)

* 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. Blood Induced Schizonticidal Tests in Rhesus Monkeys
Summary of Combination Studies

WRAIR Drug Number	Combination Ratio	Minimum Curative Dose (mg/kg/day)	Number of Monkeys (Total cured/total treated)
158122 + 180872	1 : 3.2	0.33 : 1.05	2/2
2978 + 6798	1 : 16	0.005 : 0.08	1/2
4629 + 5949	1 : 1.5	10 : 15	1/2
2978 + 448	1 : 8	0.04 : 0.32	2/2

Table 3. Summary of Radical Curative Tests in Rhesus Monkeys
Sporozoite Induced Tests

Type of Compound	WRAIR Drug Number	** Minimum Curative Dose (with 4.0 mg/kg/day of chloroquine phosphate (mg/kg/day))
8 - Aminoquinoline	4234	* NC (10.0)
	106147	3.16
	182232	3.16
	183489	3.16
	211820	10.0
Miscellaneous	14262	* NC (10.0)
	222249	* NC (10.0)
	223660	* NC (10.0)

* Not curative. The compound did not cure at the maximum dose tested.

The maximum tested dose is indicated in parentheses.

** Administered with 4.0 mg/kg/day of chloroquine phosphate.