

EVALUATION OF EXPERIMENTAL ANTIMALARIAL DRUGS FOR
RADICAL CURATIVE ACTIVITY IN THE RHESUS MONKEY

Principal Investigators : George S. Ward, LTC, VC
Richard G. Andre, MAJ, MSC
Pranee Hansukjariya, BSc.
Suwattana Vongpradist

Associate Investigator : David E. Davidson, Jr., COL, VC*

OBJECTIVE : To evaluate the radical curative effectiveness of selected experimental drugs in rhesus monkeys (*Macaca mulatta*) infected with *Plasmodium cynomolgi* malaria.

BACKGROUND : This is a continuation of studies initiated by this Laboratory in 1974. A chronological report of the methodology and results are available in previous SEATO/AFRIMS Annual Reports (1,2). These studies are conducted in association with the Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research.

METHODS : Rhesus monkeys were inoculated intravenously with sporozoites produced in *Anopheles dirus* mosquitoes. *A. dirus* mosquitoes were fed on *P. cynomolgi* infected Rhesus monkeys. This feeding was conducted during the second rise in parasitemia and when both male and female gametocytes were present as evidenced by a blood smear. On post-feeding day 14, the sporozoites were harvested from the thoraces of the infected mosquitoes and diluted in a saline-normal monkey serum solution (1:1) to a concentration of $5-20 \times 10^3$ sporozoites per ml. Pre-selected, malaria-negative rhesus monkeys were immediately inoculated intravenously with one ml of the sporozoite solution. Each monkey was monitored by blood smears daily, beginning on day 6 post-treatment for the development of a parasitemia. When the parasitemia reached 5×10^3 parasite per cmm., test drugs were administered daily for seven days at a predetermined dosage level, based on body weight. To permit evaluation of drug activity against tissue parasitic forms independent of blood schizonticidal activity, chloroquine phosphate was administered simultaneously with each test drug at 10 mg/kg body weight/day for seven days.

Following administration of the test drug, malaria parasitemia was monitored daily by examination of Giemsa-stained blood smears for 20 days and on Monday, Wednesday and Friday thereafter. Monkeys which converted to a negative parasitemia were monitored for 100 days post-treatment. Those remaining negative during this period were considered cured. Monkeys which initially converted to a negative status but became positive again within 20 days post-treatment were considered not cured. These monkeys were terminated on that particular drug study; however when their parasitemias reached an acceptable

* Division of Experimental Therapeutics, WRAIR.

level (approximately 2,000/cmm), they were placed on another test drug. In this manner, it was possible to use one monkey to test several drugs, provided they "break" with a parasitemia before post treatment day 20. Monkeys that remained negative for over 20 days post-treatment but subsequently became positive in less than 100 days were also considered not cured. These monkeys were not used in subsequent drug tests as they rarely developed a high enough parasitemia to provide an accurate measure of the effectiveness of a second drug. In these cases, the monkeys were given a combination of chloroquine at 10 mg/kg of body weight and primaquine at 1.78 mg/kg body weight for seven days. This rendered the monkeys "clean" of malaria parasites. Following this regimen, the monkeys were either issued to other departments for use in various protocols or shipped back to Walter Reed Army Institute of Research for further use by investigators there.

RESULTS : A primaquine baseline study was completed. Evaluation was then completed on seven experimental drugs. The results are summarized in Table 1. Screening tests were initiated on 20 compounds and 18 new compounds are received for evaluation. Twenty-two young rhesus were received from the U.S. for completion of testing in FY 82 and plans were completed to obtain 17 malaria naive females from Ft. Detrick as well as 30 young females from the NIH/Litton colony at Yemassee for testing in FY 83. During the testing period, the dose of the blood schizonticide chloroquine was increased from 6.2 to 10 mg/kg daily, following several failures to clear parasitemia in 7 days with the 6.2 mg dose.

Table 1. Summary of Completed Sporozoite Induced Tests in Rhesus Monkeys.

Type of Compound	WRAIR Drug Number	Minimum Curative Test Dose (mg/kg/day)
8-Aminoquinoline	249281	>1.0
	249602	1.0
	249420	0.316
	243254	1.0
Miscellaneous	250016	>10
	2494921	>10
	213640	>3.16

* Administered orally with 10.0 mg/kg/day chloroquine phosphate.

REFERENCES :

1. Brown, J.L., et al., Annual Progress Report, SEATO Medical Laboratory, April 1975 - March 1976. pp. 133-135.
2. Brown, J.L. et al., Annual Progress Report, AFRIMS, April 1976 - September 1977. pp. 155-158.