

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

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**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  $5-20 \times 10^5$  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  $5-25 \times 10^3$  parasite per cmm., test drugs were administered daily for seven days at a predetermined dosage level, based on a mg. of drug/kg of body weight. To permit evaluation of drug activity against tissue parasitic forms independently of blood schizonticidal activity, chloroquine phosphate was administered simultaneously with each test drug at 6.2 mg/kg body weight/day for seven days.

Following administration of the test drug, malaria parasitemia was monitored by examination of Giemsa-stained blood smears daily for twelve 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. Those monkeys which either failed to convert to negative parasitemia or which did convert

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to a negative status initially but subsequently became positive again before 20 days post-treatment were considered not cured. These monkeys are terminated for that particular drug study, however, when their parasitemias reach an acceptable level (approximately 5,000/cmm) they are placed in another study using a different test drug. In this manner, it is possible for one monkey to be used to test several drugs provided they "break" with a parasitemia before post treatment day 20. Monkeys that remain negative for over 20 days post-treatment but subsequently become positive in less than 100 days are also considered not cured. These monkeys are not used in subsequent drug tests as they rarely developed a parasitemia level high enough (approximately 5,000/cmm.) to provide an accurate measure of the effectiveness of a second drug. In those cases, they are given a combination of chloroquine at 6.2 mg/kg. of body weight and primaquine at 1.3 mg/kg. body weight for seven days. This renders the monkeys "clean" of malaria parasites. Following this regimen, the monkeys are 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, or used in the AFRIMS breeding colony.

**RESULTS :** Evaluation was completed on a total of 22 experimental drugs. The results are summarized in Table 1. Screening tests were initiated on 9 compounds and 10 new compounds were received for evaluation. This reduced number of drugs tested over previous years is a direct result of the world-wide shortage and increased cost of rhesus monkeys available for medical research. The rhesus monkeys that were on hand and purchased during FY 80 were utilized and then the testing was discontinued in the spring of 1981. Subsequently 29 rhesus males were purchased from the National Primate Center at Davis, California and the monkey-mosquito-monkey cycle was reestablished for continuation of the project at a decreased level in the fall of 1981. Future projections are to continue drug evaluation using approximately 50 rhesus monkeys per year depending on purchase costs and output of the AFRIMS rhesus breeding colony.

**PUBLICATION :**

Davidson, D.E., Jr., Ager A.L., Brown, J.L., Chapple, F.E., Whitemire, R.E., & Rossan R.N. : New tissue schizonticidal antimalarial drugs. Bulletin of the World Health Organization 59(3):463-479 (1981)

**REFERENCES :**

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

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

Type of Compound	WRAIR Drug Number	Minimum Curative Dose* (mg/Kg/day)
8-aminoquinoline	247705	0.1
	248412	1.0
	248747	1.0
	249259	0.316
	248811	1.0
	247067	1.0
	247066	1.0
	246315	0.316
	245543	**NC(1.0)
	245541	NC(1.0)
	245540	NC(1.0)
	244667	NC(1.0)
	244160	1.0
	244153	NC(1.0)
	243832	NC(1.0)
	243780	NC(1.0)
	242511	0.1
	242474	1.0
	242471	0.316
	242047	NC(1.0)
245542	NC(1.0)	
Miscellaneous	243270	NC(10.0)

\* Administered orally with 6.2 mg/Kg/day of Chloroquine phosphate.

\*\* Not Curative - The compound did not cure at the maximum dose tested or tolerated. The maximum dose is indicated in parentheses.