

## 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 or third 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 salivary glands of the infected mosquitoes and diluted<sup>5</sup> in a saline-normal monkey serum solution (1:1) to a concentration of 5-20 X 10<sup>5</sup> 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<sup>3</sup> for the development of a parasitemia. When the parasitemia reached 5-25 X 10<sup>3</sup> 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 80 days post-treatment. Those remaining negative during this period were considered cured. Those monkeys which either failed to convert to a negative status initially but subsequently became positive again in under 20 days post-treatment were considered not cured. These monkeys

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were terminated on that particular drug study when this occurred, however, when their parasitemias reached an acceptable level (approximately 5,000/cmm) they were placed on another, different 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. Those monkeys that remain negative for over 20 days post-treatment but subsequently become positive in less than 80 days are also considered not cured. These monkeys are not used in subsequent drug tests as they rarely develop a parasitemia level high enough (approximately 5,000/cmm.) to provide an accurate measure of the effectiveness of a second drug. In these cases, the monkeys 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 they are shipped back to Walter Reed Army Institute of Research for further use by investigators there.

RESULTS : A total of 37 experimental drugs were evaluated using 109 rhesus monkeys. The results are summarized in Tables 1 and 2. This reduced number of drugs tested over previous years is a direct result of the world-wide shortage of rhesus monkeys available for medical research. There are twenty five rhesus monkeys remaining in the animal colony which can be used in this project. This will allow us to continue the program through the end of calendar year 1980. If a new source of rhesus monkeys can not be found, this project will be terminated at that time.

There are two possibilities that this project can be continued.

1. the moritorium against the export of rhesus monkeys from India is due to expire in April 1981 according to the Indian government's original plan (the moritorium was for 3 years). If the export ban was lifted rhesus monkeys would then again be available for the continuation of the project.

2. the development of the cynomolgous monkey (*Macaca fascicularis*) as a new animal model for the evaluation of antimalarial drugs in this system. (For details see "Evaluation of *Macaca fascicularis* as a Laboratory Model for Malaria" elsewhere in this report).

#### 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	239372	1.0
	238608	3.16
	238850	**NC (3.16)
	242897	NC (1.0)
	241320	NC(10.0)
	238605	0.1
	237807	0.316
	243789	10.0
Miscellaneous	230190	NC (1.0)
	234737	NC (1.0)
	242508	NC(10.0)
	237797	0.316

\* 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.

Table 2. Summary of Drugs Currently Undergoing Sporozoite Induced Tests in Rhesus Monkeys

Type of Compound	WRAIR Drug Number	Dosage Level Currently Being Tested (mg/kg/day)
8-Aminoquinoline	242471	1.0, 0.316, 0.1
	242511	1.0, 0.316, 0.1, 0.0316
	243254	1.0, 0.316, 0.1
	244662	3.16, 1.0
	246315	10.0, 1.0, 0.1
	247067	1.0, 0.316, 0.1
	247066	1.0, 0.316, 0.1
	247705	1.0, 0.1, 0.0316
	248811	1.0, 0.1
	242047	1.0
	243780	1.0, 0.1
	243832	1.0, 0.1
	244153	1.0
	244160	1.0, 0.1
	245542	1.0
	245541	1.0
	245543	1.0
	245540	1.0, 0.1
	233099	1.0, 0.1
	248412	1.0, 0.1
248474	1.0, 0.1	
242474	1.0	
Quinoline	3863-D-0	1.0, 0.1
Miscellaneous	243270	10.0, 1.0, 0.316, 0.1
	009792	1.0