

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 the SEATO/AFRIMS Medical Research Laboratory Annual Reports, 1975-76 and 1976-77. These studies are conducted in association with the Department of Experimental Therapeutics, Walter Reed Army Institute of Research.

METHODS : Rhesus monkeys were inoculated intravenously with sporozoites produced in *Anopheles balabacensis* mosquitoes.

A. balabacensis mosquitoes were fed on *P. cynomolgi* infected 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 in a saline-normal monkey serum solution (1:1) to a concentration of $5 - 20 \times 10^5$ sporozoites per ml. Preselected, malaria-negative rhesus monkeys were immediately inoculated with one ml. of the sporozoite solution.

Each monkey was monitored by blood smears daily, beginning on day 7 post-treatment, for the development of a parasitemia. When the parasitemia reached $5 - 25 \times 10^5$ parasites per cmm, drugs were administered daily for seven days at a predetermined dosage level, based on 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 5 mg/kg body weight/day.

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. Prior to 1 March 78, monkeys which converted to a negative parasitemia and remained so through post-treatment day 20 were splenectomized and monitored an additional 33 days. Those that remained

free of malaria parasites during this period were considered cured. After 1 March 78, monkeys which converted to a negative parasitemia were monitored for 80 days post-treatment with no splenectomy. Those remaining negative during this period were considered cured.

RESULTS : A total of 79 experimental drugs were evaluated; results are summarized in Table 1.

Splenectomy of negative monkeys was discontinued in an effort to preserve the monkeys for further research. This was deemed necessary, especially in light of the ban on the exportation of rhesus monkeys from India. Also due to the ban, a reduction in the number of drugs being tested was necessary. Plans are now being formulated to investigate the possibility of other non-human primate hosts, especially the Malaysian *Macaca fascicularis* (Crab eating Macaque).

Problems encountered : The *A. balabacensis* colony was decimated apparently due to a change in diet. The mosquitoes were fed on rat and mouse chow and during the year the source of this feed was changed from the United States to a local producer. This problem was rectified by returning to the U.S. manufactured rat and mouse chow. During the mosquito to monkey sporozoite passage, SP-30, the malaria sporozoites were lost. After several unsuccessful attempts the sporozoites were recovered by the following procedure : An old sporozoite donor monkey with a low chronic parasitemia was splenectomized and when its parasitemia reached 10^5 parasites per cmm, blood was drawn and inoculated intravenously into two malaria free monkeys. These monkeys were then monitored daily and during the second parasitemia rise with male and female gametocytes present *A. balabacensis* mosquitoes were allowed to feed. The mosquitoes were handled and the sporozoites harvested and inoculated into two malaria free monkeys in the routine manner. Malaria parasitemia in these monkeys was evidence that the monkey - mosquito - monkey sporozoites had been recovered.

Table 1. Summary of sporozoite induced tests in rhesus monkeys

Type of Compound	WRAIR Drug Number	Minimum Curative Dose* (mg/kg/day)
8 - Aminoquinoline	199981	**NC (10)
	211814	1.0
	214198	NC (10)
	215730	10.0
	219373	10.0
	222890	10.0
	225374	1.0
	225448	0.316
	225845	1.0
	226261	NC (1.0)
	226899	0.316
	226984	1.0
	228000	1.0
	228002	1.0
	228335	1.0
	228583	0.316
	229406	10.0
	230395	10.0
	231030	1.0
	231033	1.0
	232584	0.316
	232956	0.316
	233078	1.0
	233195	1.0
	233537	1.0
	233539	1.0
	233627	1.0
	233821	1.0
	233878	3.16
	233881	3.16
	234099	10.0
	234578	1.0
	234738	3.16
	235202	1.0
	235485	1.0
	235720	10.0
235724	10.0	
236066	NC (10)	
236645	10.0	
236646	NC (10)	

* Administered orally with 5.0 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.

Type of Compound	WRAIR Drug Number	Minimum Curative Dose* (mg/kg/day)
Acridines	226970	NC (10)
	227282	NC (10)
	231135	NC (10)
	233599	NC (10)
	233600	NC (10)
	233602	NC (10)
	233626	NC (10)
	233744	NC (10)
	234064	NC (10)
	235471	NC (10)
	235474	NC (10)
	235477	NC (10)
Pteridines	40070	NC (10)
	236062	NC (10)
	236087	NC (10)
2-4 Diaminoquinazoline	150015	NC (10)
	150017	NC (10)
	155004	NC (10)
Naphthoquinone	25175	NC (10)
	49808	NC (10)
Quinolines	212293	NC (10)
	230688	NC (10)
Miscellaneous	6012	NC (10)
	77250	NC (10)
	96345	NC (10)
	190729	NC (10)
	194905	NC (10)
	194965	NC (10)
	203659	NC (10)
	210809	NC (10)
	228258	NC (10)
	229184	NC (10)
	232439	10.0
	233538	NC (10)
	234852	10.0
235201	NC (10)	

* Administered orally with 5.0 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.