

EVALUATION OF EXPERIMENTAL ANTIMALARIAL DRUGS FOR RADICAL CURATIVE ACTIVITY IN THE RHEBUS 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.

* Division of Experimental Therapeutics, WRAIR.

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 5×10^3 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 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 : The mosquito-monkey *P. cynomolgi* cycle has been reestablished and 45 rhesus and splenectomized cynomolgus monkeys have been used in the antimalarial drug development program during FY 83. A primaquine baseline study has been completed. Thirteen new compounds have been received for testing. Radical curative screening has been completed on 7 compounds (Table 1) and 25 compounds are currently in progress. Thirty young U.S. origin female rhesus and 17 adult malaria virgin rhesus females from USAMRIID were shipped to AFRIMS for compound screening in FY 83. In FY 84, AFRIMS produced young rhesus will be used for radical curative testing. Potentially toxic compounds are being screened in splenectomized cynomolgus also produced in the AFRIMS breeding colony.

FUTURE OBJECTIVES : To continue screening compounds and attempt to decrease present backlog of untested compounds.

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

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

Type of Compound	WRAIR Compound Number	Minimum Curative Dose (mg/kg/day)
Indoloquinoline	249696	>10.0
8-Aminoquinoline (Metabolite)	6890	>10.0
8-Aminoquinoline	249229	1.0
8-Aminoquinoline	249380	1.0
8-Aminoquinoline	249253	0.316
8-Aminoquinoline	249582	0.316
8-Aminoquinoline	249670	0.316

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