

## Evaluation of Experimental Antimalarial Drugs for Radical Curative Activity in Rhesus Monkeys

Principal Investigators :                    John L. Brown, MAJ, VC  
   Richard G. Andre, MAJ, MSC  
   Kwanyuen Lawhaswasdi, DVM

Associate Investigators :                    LTC David E. Davidson, Jr., VC  
   MAJ Frank E. Chapple, VC  
   Markpol Tingpalapong, DVM

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

**BACKGROUND :** Studies to evaluate antimalarial drugs in rhesus monkeys began in 1971. Initial projects were designed to test suppressive (blood schizonticidal) activity. In 1974 a project was initiated with the goal of establishing an intramural capability to evaluate drugs for radical curative activity (sporozoite induced testing). These are continuing studies which are conducted in association with the Division of Medicinal Chemistry, Walter Reed Army Institute of Research.

**METHODS :** Test rhesus were infected with sporozoites produced in *Anopheles balabacensis* mosquitoes. Mosquitoes four to five days old, divided into lots of 400, were starved approximately four hours, then offered a blood meal on an intact rhesus acutely infected with *P. cynomolgi*. The feeding was scheduled to coincide with (1) the second or third rise in parasitemia, and (2) the presence of both male and female gametocytes. Mosquitoes were examined on post-feed day 6 for gut oocysts; 20 to 80 per gut were considered optimal for sporozoite development. On post-feed day 13 an estimate was made of the sporozoite concentration per salivary gland pair. The following day sporozoites were harvested and diluted in saline; normal monkey serum (1:1) to a concentration of  $5-20 \times 10^7$  per ml. Each test rhesus was inoculated intravenously with 1 ml.

Parasitemia developed in eight days. After parasitemia reached  $5-25 \times 10^5$  per cmm, approximately 11 to 15 days, drugs were administered daily for seven days on the basis of mg/kg 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 5.0 mg/kg/day.

Following administration of the test drug, blood was monitored by examination of giemsa stained blood smears daily for twelve days and every two days thereafter. Monkeys which remained negative on post-treatment day 20 were splenectomized and monitored an additional 33 days. Splenectomized monkeys which remained free of malarial parasites through post-treatment day 53 were considered cured.

RESULTS : Problems encountered in sporozoite production were rectified and procedures for production and quantitation of sporozoites were standardized. Therefore, the goal of establishing a radical curative test capability was fulfilled during this report period. A total of 87 experimental drugs were evaluated, results are summarized.

Summary of Sporozoite Induced Tests

Type of Compound	WRAIR Drug Number	Minimum Curative Dose** (mg/kg/day)
8-aminoquinoline	6020	10.0
	6026	1.0
	181023	1.0
	184118	*NC (3.16)(toxic at 10.0)
	211666	3.16
	212293	NC (3.16)
	216100	3.16
	216643	NC (10.0)
	216644	NC (10.0)
	218573	NC (10.0)
	219423	NC (1.0)(toxic at 10.0)
	223442	1.0
	223658	1.0
	223747	10.0
	223756	1.0
	224097	0.316
	224382	3.16
	224398	NC (3.16)(toxic at 10.0)
	224486	1.0
	225503	NC (3.16)
	225635	NC (10.0)
	225742	NC (10.0)
	226256	1.0
	226257	NC (1.0)(toxic at 10.0)
	226292	1.0
	226296	NC (1.0)(toxic at 3.16)
	226393	10.0
	226394	10.0
	226426	0.316
	226573	1.0
	226619	3.16
	226762	10.0

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

\*\* Administered orally with 5.0 mg/kg/day of chloroquine phosphate.

Summary of Sporozoite Induced Tests (continued)

Type of Compound	WRAIR Drug Number	Minimum Curative Dose** (mg/kg/day)
8-aminoquinoline	226937	3.16
	227175	*NC (10.0)
	227495	1.0
	228327	3.16
	228456	0.316
	228457	1.0
	228708	1.0
	228710	1.0
	229431	NC (10.0)
	230388	NC (1.0) (toxic at 10.0)
	230837	NC (10.0)
	231163	10.0
	231350	NC (10.0)
	231530	0.316
	231776	10.0
	232147	NC (10.0)
Naphthyridinones	206287	NC (10.0)
	222119	NC (10.0)
	231138	10.0
	231165	NC (10.0)
	232144	NC (10.0)
	232179	NC (10.0)
Pteridines	199361	NC (10.0)
	206891	NC (10.0)
Quinazoline	222448	NC (10.0)
	225329	NC (10.0)
Quinolines	229092	NC (10.0)
	229011	NC (10.0)
	228258	NC (10.0)

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

\*\* Administered orally with 5.0 mg/kg/day of chloroquine phosphate.