

THE EFFECT OF CHLOROQUINE ON THE INFECTIVITY OF RODENT MALARIA PARASITES TO *ANOPHELES STEPHENSI**

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ABSTRACT

There was a statistically significant difference in the number of oocysts, developed in *Anopheles stephensi* which had fed on mice infected with chloroquine resistant strains (NY 151/2B and *P.y. nigeriensis*) and treated with 1 or 10 mg/kg chloroquine phosphate, when compared with the mosquitoes fed on saline injected control animals infected with the same lines. There was no significant difference between the oocyst counts in anophelines that fed on mice which were treated with chloroquine sensitive strains.

In the first experiments on rats, with *P.y. nigeriensis*, greater increase has been shown in oocyst number in mosquitoes fed on dosed rats than in control. In duplicated experiments however, enhancement was approximately double.

In chloroquine sensitive strain (ANKA), there was no statistically difference between the number of oocyst developed in chloroquine treated and control rats.

INTRODUCTION

Ramkaran and Peters¹ demonstrated that the infectivity of a chloroquine resistant strain of *Plasmodium berghei* to *Anopheles stephensi* could be enhanced by the administration of chloroquine to infected mice, whereas chloroquine given to sensitive strains of *Plasmodium berghei* prior to mosquito feeding did not enhance infectivity. This observation of Ramkaran and Peters (*loc. cit*) could, if applicable also to chloroquine resistant strains of *Plasmodium falciparum*, help to explain the apparent rapid spread of chloroquine

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resistant strains of the latter parasite through South East Asia and South America. In view of the importance of this phenomenon, and since no reports of a comparable study have been published elsewhere, the primary aim of the present project was to determine the reproducibility of Ramkaran and Peters experimental results, using the same parasite strains, after a five year interval.

As Ladda and Sprinz² reported that strains of *Plasmodium berghei* which were sensitive to chloroquine in the mouse were more refractory to the drug in the rat, it was also intended to determine whether the infectivity to mosquitoes of such strains in the rat could be enhanced by chloroquine.

MATERIALS AND METHODS

Maintenance of strains of parasites

a) Mice

18–20 grammes male albino TFW mice, kept at $24^{\circ} \pm 2^{\circ}\text{C}$, and fed on Dixon's diet modified 41/B.

Invertebrate host

b) *Anopheles stephensi*, kept in insectary at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 75% + 10% relative humidity.

Strains of rodent Malaria used

c) Parasite transmitted through *A. stephensi* at 20°C :

1. Chloroquine sensitive NY/L10 Diggins and Gregory³ and Ramkaran and Peters.
2. Chloroquine sensitive ANKA Bafort et al.⁴
3. Chloroquine resistant NY/151 Diggins and Gregory and Ramkaran and Peters.
4. Chloroquine resistant *Plasmodium yoelii nigeriensis* (NIG) Killick-Kendrick^{5, 6}. Transmitted through *Anopheles stephensi* at 25°C .

EXPERIMENTAL PROCEDURE

10 or 15 eperythrozoan free mice (TFW strain) were infected with Plasmodium by intraperitoneal inoculation of 10^7 infected red cells. The day of infection being designated D+0. The mice were randomly divided into 2 or 3 cages of 5. On D+4 of the infection blood films were made and the mice were afterwards drugged intraperitoneally with chloroquine phosphate. The drugged mice received either 1 mg/kg or 10 mg/kg chloroquine phosphate, the control cage received an intraperitoneal injection of saline. Six or 12 hours after drug administration the mice were again blood filmed

and mice (from each cage) were used to infect mosquitoes. Each mosquito pot contained approximately 40, 3–4 day old female mosquitoes. Depending upon the strain of parasite used, the mosquitoes were either maintained at $19 \pm 1^{\circ}\text{C}$ and 80% – 90% relative humidity or at $25 \pm 2^{\circ}\text{C}$ and $75\% \pm 10\%$ relative humidity. Throughout the experiment the mosquitoes were fed with 10% sucrose solution.

On the 7th day after the blood meal the mosquito midguts were dissected out into saline and oocyst counts were made. Ten female mosquitoes from each pot were dissected.

The experiments using rats were carried out in similar manner. On D+0 3–4 week old, 50–60 gram male rates were infected intraperitoneally with 2.5–5–times more infected red blood cells than the mice received. The rats were drugged and mosquitoes fed when suitable parasitaemias and gametocyte counts were obtained (this varied from D+8 to D+10 of the infection).

RESULTS

The results have been summarized in the following tables:
a) Experiments using mice NY/L10. The results shown in Table 1 were obtained from mosquitoes which fed on mice 6 to 12 hours after drug administration. There appeared to be no significant difference in the number of oocysts developed in *Anopheles stephensi* which had fed on mice treated with 1 or 10 mg/kg chloroquine. However, in the resistant strain of NY/151 the results appear to be more consistent. There is a statistically significant difference in the number of oocysts that developed in *Anopheles stephensi* that had been fed on mice which were treated with 1 or 10 mg/kg of chloroquine phosphate when compared with the counts in mosquitoes fed on saline injected animals.

There was an enhanced infectivity of NY/151 to *Anopheles stephensi* after chloroquine administration to the mice but no enhanced infectivity was obtained using NY/L10.

In Plasmodium Yeolii Nigeriensis (NIG) (The chloroquine resistant strain).

In Table 2, there is a statistically significant difference between drugged and control experiments. However, in the ANKA chloroquine sensitive strain (Table 2), there appears to be no significant difference in the number of oocysts which developed in *Anopheles stephensi* had been fed on mice treated with 1 or 10 mg/kg chloroquine when compared with numbers in mosquitoes fed on saline injected controls.

b) Experiments using rats

In *P. Yeolii nigeriensis*, the chloroquine resistant NIG strain (Table 3).

There appears to be a significant difference in the number of oocysts on the mid-guts of *A. stephensi* which had fed on infected rats after administration of chloroquine when compared with those of the mid-guts of mosquitoes which had fed on saline injected control rats. From the results in Table 3 it is apparent that a larger number of oocysts are obtained after the administration of 1 mg/kg than 10 mg/kg chloroquine.

In ANKA, chloroquine sensitive strain (Table 3).

There appears to be no significant difference in the number of oocysts that developed in *Anopheles stephensi* that had been fed on rats treated with 1 or 10 mg/kg chloroquine when compared with counts in mosquitoes fed on saline injected controls.

DISCUSSION

The primary aim of this study was to confirm the reproducibility of the results of Ramkaran and Peters on the enhancement of the infectivity of chloroquine resistant strains of *Plasmodium berghei* following chloroquine treatment.

In this study there was a statistically significant difference between the number of oocysts that developed in *Anopheles stephensi* which had fed on mice infected with chloroquine resistant strains treated 1.0 or 10 mg/kg of chloroquine phosphate, and the number of oocysts in mosquitoes fed on saline-injected control animals. However, when mosquitoes were fed on mice infected with chloroquine sensitive strains there appeared to be a significant difference between oocysts number in the drugged groups and those in the control group.

The behaviour of the strains in the rat. In the first experiments with *P.y.nigeriensis* greater increase in oocyst number was observed in mosquitoes fed on dosed rats than in controls. It was almost ten-fold increase in numbers, contrasted with the approximate doubling of oocyst number observed in the enhancement in the mouse mosquito model. In duplicated experiments however enhancement was only approximately doubled. In chloroquine sensitive strain (ANKA) in the Rat mosquito model, there was no statistically significant difference between the number of oocyst that developed in *Anopheles stephensi* which had fed on mice injected chloroquine control. It can be observed that chloroquine does not significantly affect the number of gamocytes within the drugged animals and furthermore that strains treated with 1 mg/kg chloroquine give greater oocyst number than those treated with 10 mg/kg.

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Table 1

Oocyst counts of mosquitoes fed on untreated and chloroquine treated mice (Absolute and percentage of control counts within each line)

Drug Dose	NY/L10 Sensitive	NY/151 Resistant

Table 2

Oocyst counts of mosquitoes fed on untreated and chloroquine treated mice (Absolute and percentage of control counts within each line)

Drug Dose	ANKA Sensitive	NIG Resistant
mg/kg	Oocyst counts on D+7 Mean \pm S.E. percent of control \pm S.E.	Oocyst counts on D+7 Mean \pm S.E. percent of control \pm S.E.
Control	46.0 \pm 2.6 100 \pm 5.7	109.9 \pm 18.1 100 \pm 16.5
1	45.9 \pm 2.2 99.8 \pm 4.8	218.6 \pm 38.6 198.9 \pm 35.1
10	46.2 \pm 2.5 100.4 \pm 5.4	231.7 \pm 46.4 210.8 \pm 42.2

Table 3

Oocyst counts of mosquitoes fed on untreated and chloroquine treated rats (Absolute and percentage of control counts within each line)

Drug Dose	ANKA Sensitive	NIG Resistant
mg/kg	Oocyst counts on D+7 Mean \pm S.E. percent of control \pm S.E.	Oocyst counts on D+7 Mean \pm S.E. percent of control \pm S.E.
Control	82.2 \pm 19.4 100 \pm 23.4	4.7 \pm 2.06 100 \pm 43.8
1	87.7 \pm 15.0 105.9 \pm 18.1	65.2 \pm 9.90 1387.2 \pm 210.6
10	85.3 \pm 15.8 103 \pm 19.0	27.2 \pm 9.45 578 \pm 201