SHORT-TERM EFFECT OF CHLOROQUINE ON THE INFECTIVITY OF *PLASMODIUM CHABAUDI* GAMETOCYTES

GAUTRET P.*, VOZA T.**, CHABAUD A.G.** & LANDAU I.**

Summary :

The short-term enhancing effect of chloroquine on gametocyte infectivity was investigated with *Plasmodium chabaudi chabaudi*, a synchronous parasite which is highly sensitive to chloroquine. In comparison with control groups, oocyst numbers increased in mosquitoes fed on mice 12 hours after the injection of 5 mg/kg chloroquine (180 % of controls) although it was not statistically significant. No effect was seen with 1 mg/kg chloroquine. The authors interpretation is that chloroquine impaired the schizogony, thus reducing also the release of toxic material of parasite origin which blocks gametocytes infectivity. Results of similar experiments with other rodent species of *Plasmodium* are compared and discussed in relation with the chronobiological characteristics of these parasites.

KEY WORDS : *Plasmodium chabaudi chabaudi, Anopheles stephensi,* gametocytes infectivity, chloroquine.

Résumé : EFFET À COURT TERME DE LA CHLOROQUINE SUR L'INFECTIVITÉ DES GAMÉTOCYTES DE *PLASMODIUM CHABAUDI*

L'effet à court terme de la chloroquine sur l'infectivité des gamétocytes à été évalué chez Plasmodium chabaudi chabaudi, *parasite synchrone et très sensible à la chloroquine. Par rapport aux témoins, le nombre d'oocystes est augmenté de 180 % chez les moustiques gorgés sur des souris, ayant été traitées 12 heures auparavant, par une injection de 5 mg/kg de chloroquine. La différence n'est cependant pas statistiquement significative. Aucun effet n'a été constaté pour une dose de 1 mg/kg de chloroquine. Nous interprétons ce résultat comme étant la conséquence de la réduction de la schizogonie par la chloroquine, et de ce fait des facteurs d'origine parasitaire bloquant l'infectivité des gamétocytes. Les résultats d'expériences similaires réalisées avec d'autres* Plasmodium *de rongeurs sont comparés et discutés en relation avec les caractéristiques chronobiologiques des parasites.*

MOTS CLÉS : Plasmodium chabaudi chabaudi, Anopheles stephensi, *infectivité des gamétocytes, chloroquine.*

The short-term effects of sub-curative doses of chloroquine on the gametocyte infectivity of rodent *Plasmodium* were studied using *P. berghei*, *P. yoelii* and more recently *P. vinckei petteri* by several authors (Ramkaran & Peters, 1969, 1970; Peters *et al.*, 1970; Gautret *et al.*, 2000) following the protocol originally described by Ramkaran and Peters. With the chloroquine resistant clone (151/B2) of *P. berghei* NK 65 and the resistant NS clone of *P. yoelii*, an enhancement of transmission was observed. Compared to controls, higher oocyst numbers were found in mosquitoes fed on mice which received chloroquine 12 hours prior to the blood meal. In contrast, no enhancement was evidenced with *P. vinckei petteri* uncloned drug-sensitive strain 106 HW and with *P. berghei* NK 65 sensitive clone (L/9). These observations led us (Gautret *et al.*, 2000) to the conclusion that the effect of chloroquine was restricted to chloroquine-resistant strains of *Plasmodium*. In the present paper, we investigated the short-term effect of chloroquine on the transmission of the gametocytes of *P. chabaudi chabaudi*, a synchronous parasite, which sensitivity to chloroquine is lower than that of *P. v. petteri* and higher than that of *P. berghei* strain NK65 (Beauté-Lafitte *et al.*, 1994).

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METHODS AND RESULTS

Fifteen outbreed OFI (Iffa Credo) female mice weighing 18-20 g were injected ip with 200 µl phenylhydrazine-HCl (Sigma) solution in 0.9 % NaCl (100 mg/kg) on day – 1, to induce a high reticulocytaemia. They were inoculated ip on day 0 with 5.10^6 parasitized red blood cells from a donor-mouse infected with *P. c. chabaudi* (strain 864 VD). This procedure was shown to increase *P. chabaudi* micro and macrogametocyte and oocyst numbers (Gautret *et al.*, 1996*a*). Mice were kept under artificial light from 06:00 to 18:00 hr. On day 5 post-inoculation, mice were divided into three groups and treated at 12:00 hr with sc injections of 200 µl of either distilled water, or a solution of 1 mg/kg or 5 mg/kg chloroquine diphosphate (Sigma). At this time, the predominant stage in the blood was the mid-term

trophozoite, the most sensitive to chloroquine (Cambie *et al.*, 1991) and gametocytes belonged principally to type 0 (not yet infective). The chronology of the various stages of *P. chabaudi* is detailed in Figure 1. At 12:00 hr, the predominant gametocyte stage (type 0 non infective) derived from the merozoites issued from a schizogony that occurred 36 hours before (Gautret *et al.*, 1996*a*). At 00:00 hr, laboratory bred three-six day old female *Anopheles stephensi* were allowed to feed on mice for two hours. The schizogony occurred at midnight, and type II gametocytes which are the most infective stages to mosquitoes (Gautret *et al.*, 1996*a*) were predominant. Approximately 20 mosquitoes were usually well engorged. Unfed and poorly fed mosquitoes were discarded. The remaining mosquitoes were maintained for 10 days at 24° C before dissection for oocyst counts and calculation of the percentage of infected mosquitoes. Blood smears were performed just prior to mosquito feed and stained in Giemsa stain in order to evaluate the parasitaemia (number of parasites per 100 red blood cells), schizontaemia (number of mature schizonts containing at least 12 merozoites per 100 red blood), reticulocytaemia (number of reticulocytes per 100 red blood cells) and microgametocytaemia (number of microgametocytes per 10 red blood cells). Because females are more difficult to identify only male gametocytes were counted. Gametocyte sex ratio is not modified by phenylhydrazine (Gautret *et al.*, 1996*a*). Parasitaemia, reticulocytaemia and microgametocytaemia in control mice did not differ significantly from that of the treated groups (Mann-Whitney *U*-test, $p > 0.05$) at the time of feeding, as shown in Table I. When fed on mice injected with 5 mg/kg chloroquine, mosquitoes showed a higher number of oocysts when compared to controls (180 % of controls) although

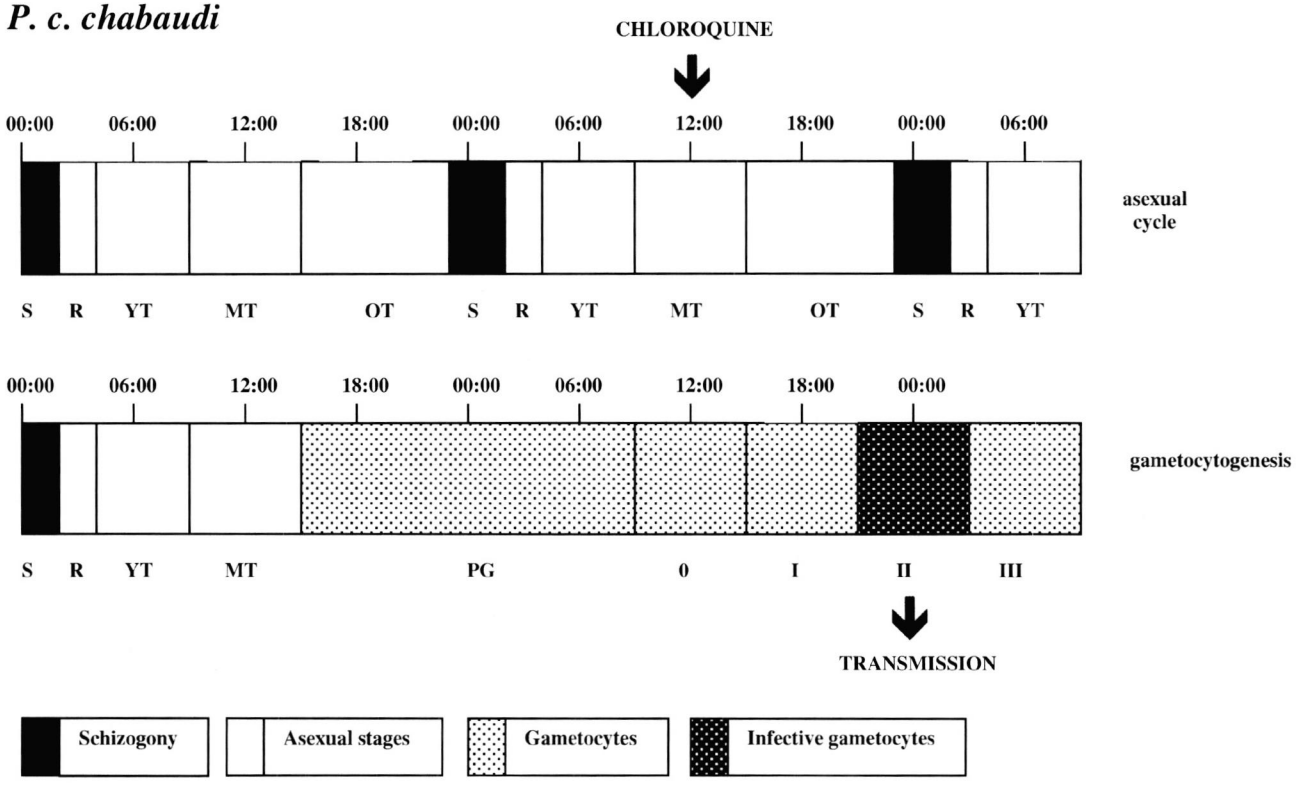

Fig. 1. – Circadian pattern of *P. c. chabaudi* blood stages and timing of experiments.
S, schizogony; R, ring; YT, young trophozoite; MT, mid-term trophozoite; OT, old trophozoite; PG, pre-gametocyte; 0, type 0 gametocyte; I, type I gametocyte; II, type II gametocyte; III, type III gametocyte.

	Schizontaemia*	Parasitaemia	Reticulocytaemia	Gametocytaemia
Controls	1.4 ± 0.9	29.7 ± 6	8.3 ± 1.7	77.3 ± 25.4
1 mg/kg chloroquine	2.0 ± 0.8	35.5 ± 7.1	8.6 ± 1.9	100.8 ± 32
5 mg/kg chloroquine	0.8 ± 0.6	28.7 ± 9.6	7.5 ± 1.5	95.2 ± 35.9

* Schizontaemia, number of mature schizonts per 100 red blood cells; parasitaemia, percentage of infected red blood cells; reticulocytaemia, percentage of reticulocytes; microgametocytaemia, number of microgametocytes per 10^5 red blood cells.

Table I. – Effect of chloroquine on *P. c. chabaudi* blood stages (mean values ± standard deviation).

	Percentage of infected mosquitoes	Mean oocysts numbers/mosquito	Mean oocysts percent of controls
Controls	77 ± 12	57.4 ± 56.9	–
1 mg/kg chloroquine	86 ± 16	56.9 ± 35.7	99 %
5 mg/kg chloroquine	96 ± 6	103.4 ± 74.0	180 %

Table II. – Effect of chloroquine on *P. c. chabaudi* transmission (mean values ± standard deviation).

the difference was not statistically significant ($p > 0.05$) and the percentage of infected mosquitoes was significantly higher ($p = 0.0262$). Injection of 1 mg/kg chloroquine did not significantly modify the oocyst numbers and percentage of infected mosquitoes ($p > 0.05$). Details are given in Table II.

DISCUSSION

Our results with *P. chabaudi* show that subtherapeutic treatment with 5 mg/kg chloroquine increases the gametocytes infectivity, 12 hours post-treatment in a dose-dependent manner. We believe that it is the consequence of an inhibition of the schizogony and of the release of factors blocking the gametocytes infectivity. Buckling *et al.* (1997) and Buckling & Read (1999) also observed a rise of infectivity of *P. chabaudi* six days after treatment which was interpreted as a reduction of crisis inhibitory factors. Chronobiological data on duration and timing of the different sexual and asexual stages of murine *Plasmodium* are essential to understand the differences between the effect of chloroquine on two synchronous and drug-sensitive species, *P. vinckei* and *P. chabaudi*. Motard *et al.* (1990; 1993) showed that the infectivity of *P. v. petteri* gametocytes was temporarily inhibited during schizogony. The peak infectivity of gametocytes of this subspecies occurs 12 hours after schizogony when type II gametocytes reach maturity and are infective, as shown in Figure 2 (Gautret *et al.*, 1996*b*). Thus, chloroquine, when given 12 hours before the peak of infectivity, does not enhance the infectivity of gametocytes as already demonstrated by Gautret *et al.* (2000). *Plasmodium chabaudi* differs from *P. vinckei*: schizogony, peak gametocytes exflagellation and maturation of infective

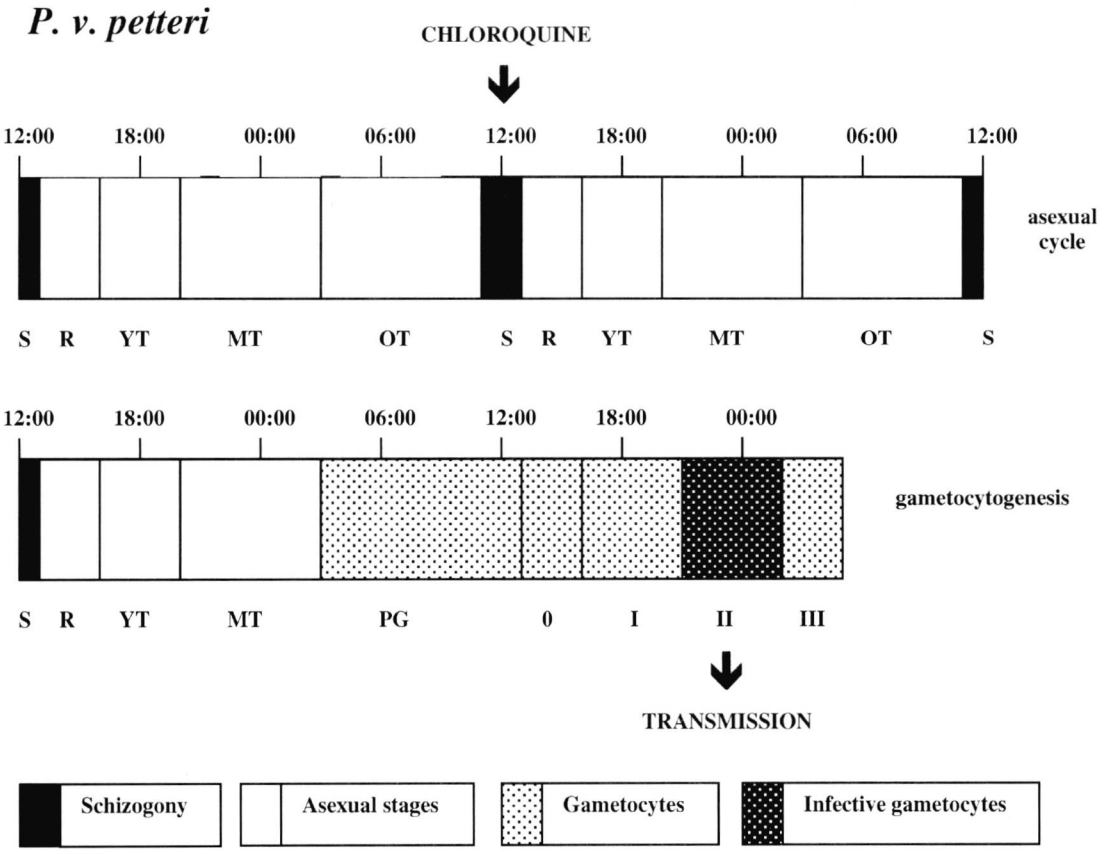

Fig. 2. – Circadian pattern of *P. v. petteri* blood stages and timing of experiments.
S, schizogony; R, ring; YT, young trophozoite; MT, mid-term trophozoite; OT, old trophozoite; PG, pre-gametocyte; 0, type 0 gametocyte; I, type I gametocyte; II, type II gametocyte; III, type III gametocyte.

gametocytes occur simultaneously around midnight (Hawking *et al.*, 1972; Gautret *et al.*, 1996*a*). No peak of infectivity was observed during the circadian cycle and it was suggested that schizogony, occurring at the time of the peak of infective gametocytes, partially inhibits infectivity (Gautret *et al.*, 1996*a*). In this work, the injection of 5 mg/kg chloroquine, when mid-term trophozoites predominate in the blood, slightly decreased schizogony (see Table I) as a consequence of the destruction of part of the trophozoites which therefore did not transform into schizonts. The infectivity of gametocytes increased accordingly. In the present experiment, the decrease of the number of circulating schizonts in 5 mg/kg chloroquine treated mice compared to untreated control mice were not statistically significant ($p > 0.05$) due to the fact that most of *P. chabaudi* schizonts sequester in the deep capillaries where schizontaemia is higher than in the circulation (Mota *et al.*, 2000). In a previous work, chloroquine has been demonstrated to lower dramatically *P. chabaudi* parasitaemia by 24 hours, when triggering mid-term trophozoites with a 5 mg/kg single dose. The parasitaemia was shown to decrease by one half in treated mice when a seven fold improvement was observed in untreated control mice (Tahar *et al.*, 1995). Alternatively to a reduction in the release of gametocyte infectivity blocking material, chloroquine could also had a direct effect on the gametocytes or on the oocysts in mosquitoes, as part of the drug is ingested in the blood meal. In our experiments, gametocyte production was enhanced by a phenylhydrazine pretreatement. It cannot be excluded that this experimental condition influenced the reaction of gametocytes to chloroquine itself or to harmful substances released by rupturing schizonts.

Whatever the mechanism involved, it is often admitted, and we have also believed that chloroquine only increased the gametocytes infectivity of resistant strains. However, our present results contradict the latter assertion by evidencing the enhancing effect of chloroquine on the infectivity of a drug-sensitive strain. Work by Ichimori *et al.* (1990) on *P. y. nigeriensis* N67 strain did not evidence a gametocyte transmission enhancement by chloroquine of neither a resistant nor a sensitive clone, while an enhancement was seen with the uncloned strain and the authors conclusion was that "there is no necessary causal connection between chloroquine resistance and the enhancement of infectivity by the drug".

The mechanism increasing or decreasing the infectivity appears to be very complex and involves many chronobiological factors: synchronicity or asynchronicity, duration of the schizogonic cycle, time of maturation of the infective gametocytes and it is not surprising to observe results apparently contradictory.

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