Influence of Sulfalene upon Gametocytogenesis of *Plasmodium falciparum* and Subsequent Infection Patterns in Anopheles stephensi

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McCARTHY, VINCENT C., AND CLYDE, DAVID F. 1973. Influence of sulfalene upon gametocytogenesis of *Plasmodium falciparum* and subsequent infection patterns in *Anopheles stephensi*. Experimental Parasitology 00, 000-000. Quinine and/or sulfalene were administered to non-immune volunteers during various stages of infection with *Plasmodium falciparum*. Administration of each drug early in the asexual parasitemia reduced or prevented the subsequent formation of gametocytes. Later administration of sulfalene produced a subsequent sterilizing effect upon immature, non-circulating gametocytes, which were non-infective to *Anopheles stephensi* when they appeared in the circulating blood. Gametocytes present in the peripheral circulation at the time of administration of both drugs or appearing shortly thereafter were infective to *A. stephensi*.

INDEX DESCRIPTORS: *Plasmodium falciparum*; *Anopheles stephensi*; Quinine; Sulfalene; Drug resistance; Gametocytogenesis; Syngamy; Sporogony; Schizogony; Life cycles.

INTRODUCTION

In the course of studies of the antimalarial properties of sulfalene (Clyde et al. 1971), special attention was given to the gametocytocidal and sporontocidal action of this sulfonamide on several strains of *Plasmodium falciparum*. Previous observations on the action of other sulfonamides on *P. falciparum* had been inconsistent. Findlay et al. (1946) found that 7 of these compounds had no gametocytocidal action, and questioned an effect reported by Durand (1939). Slute and Maryon (1951) noted that 10 to 14 days after the administration of sulfamethazine a large number of gametocytes often appeared in the blood. Powell and his colleagues (1968) noted a similar effect 5 days after the start of sulfoxon treatment; however they concluded that DDS, sulfadiazine or closely related compounds did not show promise as gametocytocidal or sporontocidal agents.

Laing (1965) found that sulforthomidine affected neither the production nor the morphologic features of the gametocytes, but postulated that the drug might render gametocytes incapable of development in the mosquito host. In a similar vein Rieckmann et al. (1968), using combinations of sulfonamides with other compounds, concluded that infectivity to mosquitoes of gametocytes emerging after initiation of treatment with one of or a combination of these agents may differ from the infectivity of gametocytes that were present in mature form before initiation of the treatment.

In this paper we intend to show that these varying views are not necessarily contradictory, and that with *P. falciparum* a
distinct pattern of gametocytogenesis in man, and subsequent infectivity or non-infectivity in mosquitoes, may be related to the stage of the parasite cycle existing in the patient at the time of treatment with a particular sulfonamide, sulfalene.

**Materials and Methods**

**Volunteers**

Informed volunteers, adult male inmates of the Maryland House of Correction, Jessup, Maryland, were selected for the study after detailed examinations for mental and physical fitness that included chest radiography, electrocardiogram, and laboratory tests. While these men may be regarded as nonimmune, some had had brief previous episodes of malaria and consequently may have acquired a slight degree of immunity. A particular prerequisite was normality of the baseline blood picture and hepatic and renal function tests. Infections were initiated either by the intravenous inoculation of 5 to 8 ml of parasitized blood or by challenge with infected mosquitoes. To prevent the possible transfer of hepatitis virus, volunteers receiving parasitized blood were given immune serum globulin intramuscularly, and the donors of the blood were examined to insure normality of liver function and absence of Australia antigen.

Blood films were made daily or more frequently if the condition warranted, and were stained with Giemsa. Patients were admitted to the hospital ward, either on clinical grounds or when parasitemia appeared, and were examined at least daily by physicians. In addition to such symptomatic treatment as was required for the comfort of the patients, small doses of quinine were given to avoid high levels of parasitemia and potentially dangerous complications. Upon completion of the study, or earlier if desired by the volunteer or considered clinically necessary, curative treatment was undertaken and was successful in all cases, patients being followed for at least 60 days. Natural reinfection of the volunteers may be excluded because malaria is not transmitted in the area of the study. The strictest attention was paid at all times to the ethical aspects of the study, these being supervised by an independent committee of senior faculty members.

**Antimalarial Drugs**

Ingestion of the sulfalene and quinine used in this study was carefully supervised by the nursing staff. On the rare occasions when the drug was vomited the treatment was repeated.

**Malaria Parasites**

Four strains of *P. falciparum* were responsible for the infections treated in this study. The McLendon strain is sensitive to chloroquine, pyrimethamine, and chlorguanide (proguanil). The Thailand Man., Malaya Tay., and Vietnam Smith strains are resistant to chloroquine and pyrimethamine when these drugs are used curatively or prophylactically. The Tay. strain, unlike the Smith strain, is sensitive to chloroguanide used prophylactically.

**Mosquito Vector**

*Anopheles stephensi* has successfully transmitted the four aforementioned malarial strains on more than 70 occasions. This mosquito line was originally collected in New Delhi and supplied to us by Drs. George Davidson and Eugene Gerberg.

Test mosquitoes were fed on suitable volunteers, then maintained at 27°C on a 5% sugar solution and water. Infection percentages were determined by examination of the mosquito mid gut, 5 to 12 days after the feed, for oocytes, and/or the salivary glands for sporozoites.
RESULTS

For clinical reasons small doses of quinine were often administered to the patients before or with the sulfalene. It is therefore necessary first to consider a case in which quinine was given alone, and then examine the effect of the addition to this treatment of sulfalene in four other cases.

1. Effect of small doses of quinine. Typical of the pattern of falciparum infection controlled but not cleared by the use of small doses of quinine is the case shown in Fig. 1, volunteer EM. Gametocytes appeared on the eighth day of parasite patency, increased in number until day 18, then slowly decreased. Mosquito infectivity commenced at a low level on day 14, peaked on day 17 and then declined.

Comment. These data fit the theory that gametocytes are initiated in the early asexual cycles but do not become identifiable in the peripheral circulation until 7-10 days later. Moreover these circulating gametocytes are not immediately infective to mosquitoes (N.B. day 13 feeding). We have obtained highest mosquito infection results when the gametocyte count has exceeded 200 per mm$^3$ for three consecutive days, thus allowing the gametocytes at least one full cyclic period (48 hrs) in the peripheral circulation.

Early in gametocytemia the gametocyte count on any given day roughly parallels the asexual count 7-10 days preceding, indicating continual gametocyte production from the asexual cycle, and suggesting (once their count has peaked) their steady removal from the circulation as well.

In this case it is evident that quinine has little if any effect on mature circulating gametocytes. However, many investigators have shown that early curative treatment of asexual parasitemia with quinine will prevent the subsequent gametocytemia from reaching levels sufficient to infect mosquitoes, if indeed the gametocytes appear at all. Similarly, early curative treatment with sulfalene clears the asexual parasitemia within 48 to 72 hours and prevents the subsequent formation of infective gametocytes.

2. Effect of sulfalene given late in an infection. The graphs shown in Fig. 2, volunteer CB, illustrate the effect of 250 mg sulfalene when given late in the course of an infection, at a time when gametocytes are already in the peripheral circulation. Asexual parasitemia was rapidly cleared but the infectivity of the circulating gametocytes appeared unaffected. The gametocyte level remained constant until 10 days after the volunteer ingested the sulfalene, when a steady decline commenced.
ASEXUAL PARASITEMIA
(PER mm$^3$)

GAMETOCYTEMIA
(PER mm$^3$)

PATENCY 5
10
15
MOSQUITO FEEDING
NO. INFECTED/NO. DISSECTED

FIG. 3. Thailand Man. falciparum cured by sulfalene administered on day 3 of patency.

Comment. The decline of gametocytes indicated that young forms were no longer entering the peripheral circulation. This decline was paralleled by a decline in mosquito infectivity, until by Day 19 after the treatment with sulfalene the gametocytes no longer infected mosquitoes. However it should be noted that at least some gametocytes remained infective to mosquitoes as late as 15 days after sulfalene treatment.

3. Effect of sulfalene given early in an infection. The graphs shown in Fig. 3 represent the results of treatment of volunteer GB with 250 mg sulfalene on Day 3 of parasitemia. The trophozoites were cleared rapidly, but gametocytes appeared 6 days after the administration of sulfalene. The gametocytemia peaked 12 days after treatment of the volunteer with sulfalene and then decreased rapidly. Mosquitoes fed 8 and 11 days after the volunteer ingested sulfalene failed to become infected.

Comment. Although the lack of infection in the first group of mosquitoes could be explained by possible immaturity of the gametocytes, this does not explain the non-infection of the second group which fed 3 days later. Here it would appear that sulfalene not only prevented the later formation of gametocytes by destroying their asexual precursors, but also sterilized those gametocytes which were in the earlier stages of their development, in this case the gametocytes that started to form in the first three days of asexual parasitemia. The gametocyte levels in this case are somewhat higher than one would expect from the relatively low level and brief duration of asexual parasitemia. This effect is more noticeable when trimethoprim is used with sulfalene. In several of these cases the gametocyte counts rose into the thousands and in one case exceeded 10,000 gametocytes per mm$^3$. Mosquitoes feeding on these gametocytes almost invariably failed to become infected.

4. Effect of sulfalene given late in an infection modified by quinine. Fig. 4 illustrates a partial sulfalene effect in a patent, volunteer WM, receiving the drug after his infection had been established for several days but had been controlled by the use of small doses of quinine. The limitation in density of the asexual stages was reflected in a suppression of early gametocyte production. Sulfalene was administered on Day 14 and reduced the asexual parasitemia to 0 by Day 18. The first surge of gametocytes commenced on Day 18 also, and was related to the asexual waves of Days 10–11 (from past observations we have noted that the initiation of a substan-
tial gametocyte wave [200+] usually requires a precursor asexual wave with a density greater than 1000 parasites per mm$^3$). This asexual wave appeared 3-4 days before sulfalene was given, and the gametocytes formed from it were infective to mosquitoes from Day 22 to at least Day 24. However, the subsequent asexual waves gave rise to sterile gametocytes. Thus, as the first gametocyte wave lost its infectivity, mosquito feedings became negative (Days 27-34).

Comment. It would appear from this that the period of infectivity of any particular wave of gametocytes is brief, probably a matter of a few days, and that infectivity over a period of many days is the result of the overlap of successive infective gametocyte waves.

5. Effect of sulfalene given with quinine at a time of high trophozoite density. The data shown in Fig. 5, volunteer LM, are in many respects similar to those in Fig. 4. Here, however, administration of sulfalene on Day 7 reduced the high level of asexual parasitemia markedly but failed to eliminate it. The first gametocytes appeared on Day 9. The first major gametocyte waves (from days 3-4) appeared in the circulation on Days 11-14 and were infective to mosquitoes on Day 17. Later waves were sterile and, as the first wave of gametocytes became non-infective or disappeared from the circulation, the mosquito feedings also became negative with the exception of a single mosquito feeding on Day 24.

Comment. Similar low levels of mosquito infection were observed in two other cases where sulfalene failed to clear the asexual parasitemia. Evidently in these cases gametocytogenesis proceeds at a low level from those asexual parasites that survive the drug, and in turn these gametocytes are unaffected by the sulfalene. However the true concentration of these potentially infectious gametocytes cannot be estimated, due to the continued presence in the blood of many sterilized gametocytes.

DISCUSSION

It is impossible, in a human volunteer system, to allow the asexual parasitemia necessary to engender gametocyte formation to persist untreated. Therefore in each case some drug medication was required (usually before the onset of gametocytemia). Quinine was selected as standard medication in these cases as its therapeutic action is best known and because small doses appear to have no noticeable effect upon mature gametocytes. However, as Mackerras and Ercole (1949) pointed out, there remains the possibility that even partial quinine treatment may damage early developing gametocytes or their precursors. By comparison the action of sulfalene upon both the asexual stages and the early stages of gametocytogenesis is clear and distinct. It may be that the effect of a drug upon the asexual stages of the parasite is also related to the effect that it will have upon the early stages of gametocytogenesis. Small doses of quinine have only a partial effect upon
asexual parasitemia and on early forming gametocytes, whereas small doses of sulfalene have a virtually total effect upon both.

Hawking et al. (1971) proposed a model for gametocytogenesis and syngamy which in some aspects agrees with our findings. They postulated a 12 day period for gametocyte development to infectivity. This fits closely with our own observations. However, they limited the infectivity of a gametocyte wave to a few hours, the limiting factor being the ability of the microgametocyte to exflagellate. Our observations suggest the infectivity of a gametocyte wave for several days. We previously noted that gametocytes remained infective to mosquitoes 15 days after successful sulfalene treatment. As sulfalene also affects the early forming gametocytes, the last gametocytes to escape the sulfalene would have been three days into their development, or at the time of successful mosquito feeding 18 days old. If a wave of gametocytes became infective on Day 12, this would indicate an infectivity span of 6 days.

The possibility of diurnal-nocturnal variation in the mosquito infection of P. falciparum has been suggested. As yet we have seen no evidence for this in our work. The bulk of our mosquito feedings took place at mid-day, but on a few occasions a series of day-night feedings were carried out on individual volunteers. Those gametocyte carriers that failed to infect mosquitoes in day time failed also at night, and those that infected mosquitoes showed no measurable variation in infectability between day and night.

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