The evolution of tafenoquine—antimalarial for a new millennium?

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Over the past three decades the emergence and geographical dissemination of drug resistance has hampered both the treatment of acute malaria and its prevention by chemoprophylaxis. The need to cure established infections in indigenous people in endemic areas continues to present a global challenge, but a particular difficulty arises at present from the exponentially increasing numbers of non-immune people who travel to malarious countries. *Plasmodium falciparum*, the parasite causing malignant tertian malaria, is the main cause of morbidity and mortality and is responsible for the great majority of deaths on the African continent, as well as in travellers returning to non-endemic countries. Between 0.43 and 0.86 million African infants up to four years of age die of malaria every year[1]. Drug resistance in this parasite first emerged in the early 1960s when chloroquine, until then the most widely used, safest and cheapest blood schizontocide, failed to cure acute infections. Since that time, a pattern of multidrug resistance has evolved and has now reached a critical point. *P. vivax*, which accounts for considerable morbidity though few deaths, is characterized by the development of relapses from the latent stage of the parasite in the liver, the hypnozoite (Figure 1). Radical cure by destroying the hypnozoites can be achieved only by administering a lengthy course of primaquine, an 8-aminoquinoline which can have serious side-effects. Moreover, chloroquine resistance has appeared in *P. vivax* in several countries. The need for new antimalarials is now acute, but until very recently most of the pharmaceutical industry has responded in lukewarm fashion at best. This paper focuses on the evolution of a novel 8-aminoquinoline that has emerged from the antimalarial drug programme first started by the Walter Reed Army Institute of Research (WRAIR) in the USA in 1963. Originally labelled WR 238,605 it was later called etaquine and has now been given the generic name tafenoquine. The potential role of this broad-spectrum compound is discussed from two points of view—as a long-acting chemoprophylactic for non-immune travellers, and as an agent for the treatment of established infection with multidrug-resistant *P. falciparum* or the radical cure of *P. vivax*.

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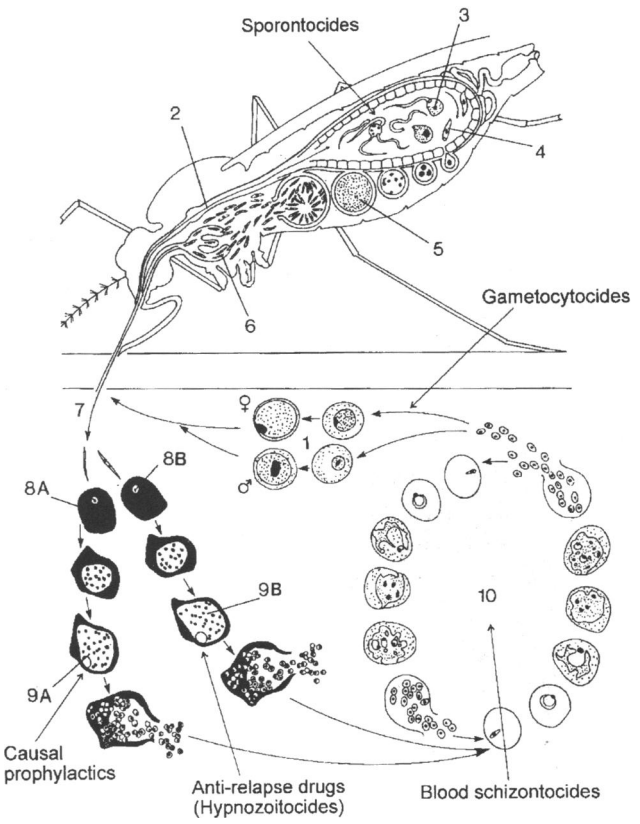

Figure 1 **Life cycle of *Plasmodium vivax* and stages on which different types of drugs act.** 1, gametocytes; 2–6, sporogonic stages; 7, infective sporozoites; 8A 9A, pre-erythrocytic schizogony; 8B 9B, secondary exoerythrocytic schizogony from hypnozoites; 10, asexual intraerythrocytic schizogony. (Based on an original figure by Andrea Darlow)

BACKGROUND

The first synthetic antimalarial drugs

From the time of its introduction to Europe from Peru sometime in the early seventeenth century[2], quinine had been the sole drug that was known in the Old World to relieve the symptoms of the intermittent fevers. Quinine, however, was poorly tolerated and did not completely cure all types of malaria infection. On 21 June 1891, the German chemist Paul Ehrlich and his physician colleague Paul Guttman took the heroic step of giving methylene

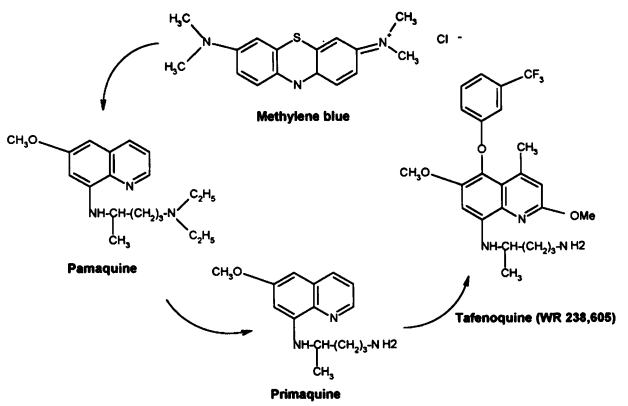

Figure 2 **Evolution of WR 238,605 (tafenoquine) from methylene blue**

blue, a synthetic dye known to stain living malaria parasites in the blood, to a young man with tertian malaria. The following month Guttman and Ehrlich treated a second patient, a 57-year-old seaman who seems in retrospect to have had a late relapse of an old malaria infection, with a 14-day course of methylene blue; he too survived. The case reports appeared in the *Berliner Klinische Wochenschrift* for 28 September 1891, some six years before Ross showed that malaria is transmitted by anopheline mosquitoes. Their article concluded with a prophetic suggestion that (my translation) 'Moreover, the important question needs to be resolved of whether a combination of quinine with methylene blue could cure infection with the tropical form of malaria [i.e. *P. falciparum*] which so frequently resists treatment with quinine alone.' We will return to the theme of drug combinations later.

Although the value of methylene blue was later confirmed by other physicians—for example, in German labourers infected with quinine-refractory *P. falciparum* in Brazil[4,5]—it was not until after the First World War, when quinine was very scarce, that a major drive was made to discover more active and less toxic drugs. From the point of view of controlling malaria it was desirable to find a compound that would, unlike quinine, destroy gametocytes and thus interrupt transmission. The first compounds were derived by Werner Schulemann and his colleagues in the Farbenfabriken Bayer in Elberfeld from methylene blue, by substituting a diethylaminoethyl group for one of the methyl groups[6,7], but none was satisfactory. They therefore started to synthesize new compounds based on the structure of quinine. Among over 12 000 compounds examined was 6-methoxy-8-aminoquinoline, later named plasmochin (also called plasmoquine or pamaquine), synthesized in 1924 by Schönhöfer under the direction of Hörlein[8]. Its exceptional potency against the blood stages of avian malaria was identified by Roehl[9]. Early clinical trials in neurosyphilitic patients being treated with induced falciparum or vivax malaria revealed, however, that pamaquine (Figure 2) was less effective than hoped in curing blood-induced infections—i.e. it was a poor blood schizontocide[10]. However, it subsequently transpired that, especially in conjunction with quinine, pamaquine did destroy gametocytes (Figure 1) and was in addition very effective in preventing the relapses of infection with *P. vivax* that we now recognize are due to persisting hypnozoites in the liver. It was also found to act as a causal prophylactic[11], though far too toxic to be used for this purpose.

The emergence of primaquine

The need for a safer anti-relapse drug against *P. vivax* provided the stimulus, during the Second World War, for the synthesis and evaluation of new series of 8-aminoquinolines. This work was conducted mainly in the USA but also in France and the USSR. However, the experimental models then available as primary screens in America, mainly *P. gallinaceum* in chicks[12] or *P. lophurae* in ducks, were not adequate for making a comparative assessment between the different new compounds. In the event, some 93 compounds were evaluated in American prison volunteers. Most of these trials were made in infections with the New Guinea Chesson strain of *P. vivax* that was isolated during the Second World War. This strain later proved to be relatively insensitive to 8-aminoquinolines[13]. Later animal studies by Schmidt and his colleagues[14] opened the way to the investigation of the anti-relapse properties of drugs in rhesus monkeys (infected with *P. cynomolgi*), avoiding the need for volunteers. From the very large number that were synthesized, two novel 8-aminoquinolines were identified, in the US programme, that were more effective and less toxic than pamaquine—namely, pentaquine and isopentaquine—and these in turn were soon replaced by an even safer compound, primaquine

Table 1 **Problems with primaquine**

Type of problem	*Manifestation*
Toxicity to man (*d*-isomer < *l*-isomer)	Haemolysis in presence of G-6-PD and some other enzyme deficiencies Methaemoglobinaemia Gastrointestinal symptoms Toxicity limits use as causal prophylactic or as blood schizontocide
Metabolism	Short half-life because of rapid metabolism
Variable drug response	Hypnozoites relatively insensitive, e.g. SW Pacific

(SN-13,272) (Figure 2), which was synthesized by Elderfield and his associates[15]. In the USSR, quinocide (which had also been synthesized in the US programme but discarded because of toxicity) was manufactured and used for some time in place of primaquine[7,16].

In 1950 primaquine was pronounced to be the drug of choice for the radical cure of vivax malaria[17]. Primaquine, however, like all its predecessors, was by no means non-toxic (Table 1). Among the serious side-effects it could produce were acute haemolysis (which was later shown to be associated especially with certain types of glucose 6-phosphate dehydrogenase deficiency), methaemoglobinaemia and severe gastrointestinal disturbances[18]. Primaquine, like pamaquine, proved to act not only on the relapsing stages of *P. vivax* but also on its primary or pre-erythrocytic liver stages (Figure 3 and Table 2); in addition it probably acted on the same stages of other *Plasmodium* species in the liver.

Although it also possessed some activity against the asexual, intraerythrocytic stages of *P. falciparum*, the level of activity was not nearly as great as that of safer compounds such as chloroquine. Now, however, studies in Thailand and West Irian (Irian Jaya) have shown that the asexual blood stages of *P. vivax* are in fact more sensitive to the action of primaquine than those of *P. falciparum* (see below). As was found for its predecessor, primaquine also has a marked gametocytocidal action, especially against *P. falciparum*. This property has been widely exploited in the attempted reduction of transmission of multiple-drug-resistant *P. falciparum* by the administration of single 45 mg doses of primaquine together with blood schizontocidal drugs such as chloroquine in endemic areas. Some have suggested that primaquine also has a direct sporontocidal action against malaria parasites in the anopheline[19], including the mature sporozoites[20]; however, it is difficult to distinguish between a sterilizing action on gametocytes and activity against the stages developing in the insect[21].

The deployment of primaquine (Table 3) took on a new dimension with the need to produce a radical cure of the

Table 2 **Stages of the malaria life cycle affected by primaquine (see Figure 1)**

Stage and location	***Species of* Plasmodium**	***Activity***
Mosquito		
Oocysts	?All	—
Sporozoite	?All	?+
Liver		
Pre-erythrocytic schizonts	*P. yoelii, P. vivax, P. falciparum*	+
Hypnozoites	*P. cynomolgi, P. vivax*	+
Asexual stages in erythrocytes	?All	+
Gametocytes in erythrocytes	?All	+

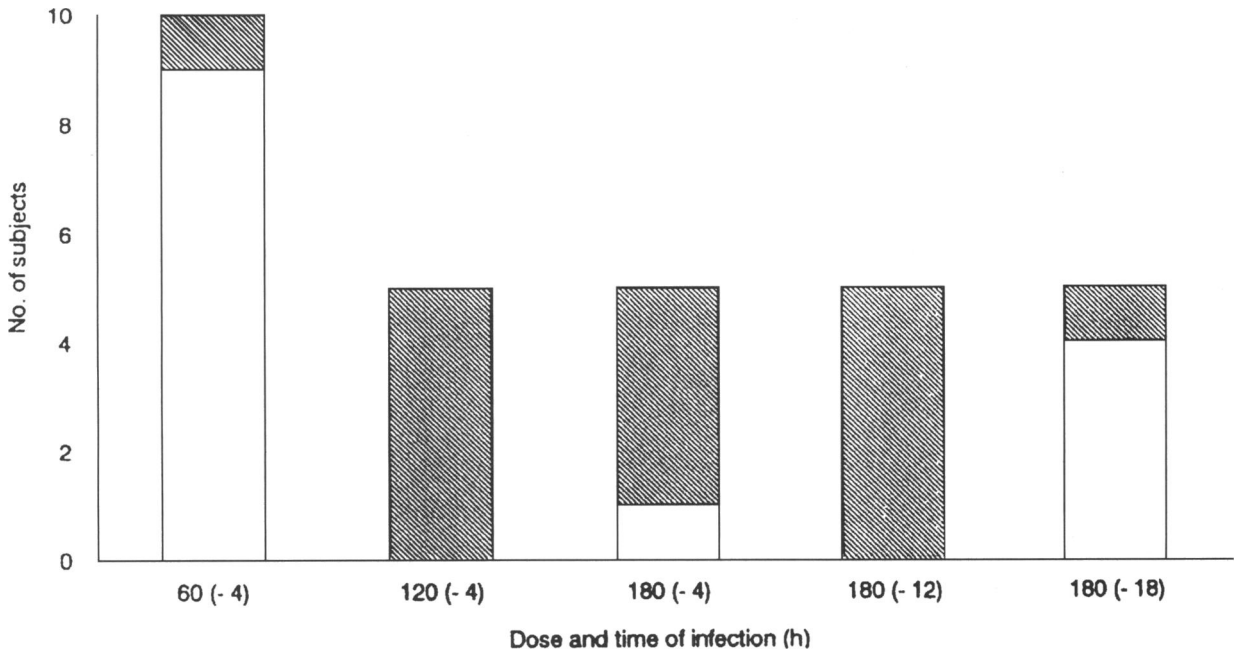

Figure 3 **Causal prophylactic action of primaquine against Chesson strain *P. vivax* in volunteers.** Single oral doses of primaquine diphosphate (mg) given at times (shown in brackets) prior to sporozoite inoculation (adapted from Ref. 10, based on unpublished data from Dr Robin Powell). □ Not protected; ▨ protected

Table 3 **Deployment of primaquine (PQ) alone or with chloroquine (CQ)***

Species of Plasmodium	Indication	Dosage regimen
P. falciparum, *P. vivax*	Prophylaxis, suppressive cure	PQ 45 mg+CQ 300 mg (CP tablet) once weekly†
P. vivax, P. ovale	Radical cure	PQ 15 mg/day × 14 days or 45 mg/week × 8 weeks
P. falciparum	Gametocytocidal action	45 mg single dose with CQ or amodiaquine treatment course

*Doses as base
†Successful in Korea but failed in Vietnam

large numbers of vivax infections in American and other troops who were engaged in the war in Korea in the early 1950s. While chloroquine was readily available for use as a suppressive prophylactic, relapses of vivax malaria were common in repatriated soldiers and these, in some cases, led to the establishment of small foci of local infection in areas of the USA that had for long been free of malaria transmission[22].

The standard 14-day course of 15 mg primaquine base per day that had been shown to be most effective in preventing relapses could not be advised because of the danger of inducing acute haemolysis in subjects with previously unidentified deficiency of G-6-PD. However, clinical studies in volunteers showed that, in place of the 14-day course, eight once-weekly doses of 45 mg primaquine base resulted in radical cure of a high proportion of those infected with Korean *P. vivax*[23]. Subsequently, a tablet combining 300 mg chloroquine base with 45 mg primaquine base was developed, the so-called CP tablet, which was later to be used both for prophylaxis and for post-exposure treatment.

At that time, because of the bad reputation of its precursor pamaquine, primaquine was still considered too toxic to be used as a causal prophylactic, even though trials in volunteers had demonstrated that well-tolerated single doses of primaquine could protect against even the primaquine-refractory Chesson strain of *P. vivax*[10] or the Panama P-F-6 (El Limon) strain of *P. falciparum*[24]. Remarkably, the latter study[24] seems to have been the only trial of primaquine as a blood schizontocide against *P. falciparum*. Either 30 or 45 mg of primaquine base daily gave inconsistent results in volunteers infected with the P-F-6 strain. In a solitary study in non-immune volunteers infected with the Chesson strain of *P. vivax*, Arnold and his co-workers[25] observed that the asexual intraerythrocytic parasites readily became resistant to primaquine in successive blood passages. From an initial regimen of 22.5 mg primaquine base daily for 7–14 days, the parasites (and, remarkably, also the patients) were able to tolerate 120 mg daily for 10 to as many as 28 days. However, the gametocytes of this, the 'Marvel' strain, had probably remained almost totally sensitive to the drug. When chloroquine resistance first emerged in South Asian strains of *P. falciparum* at the time of the war in Vietnam, the CP tablet did not provide adequate protection[26] and the massive drug development programme of the US Army was

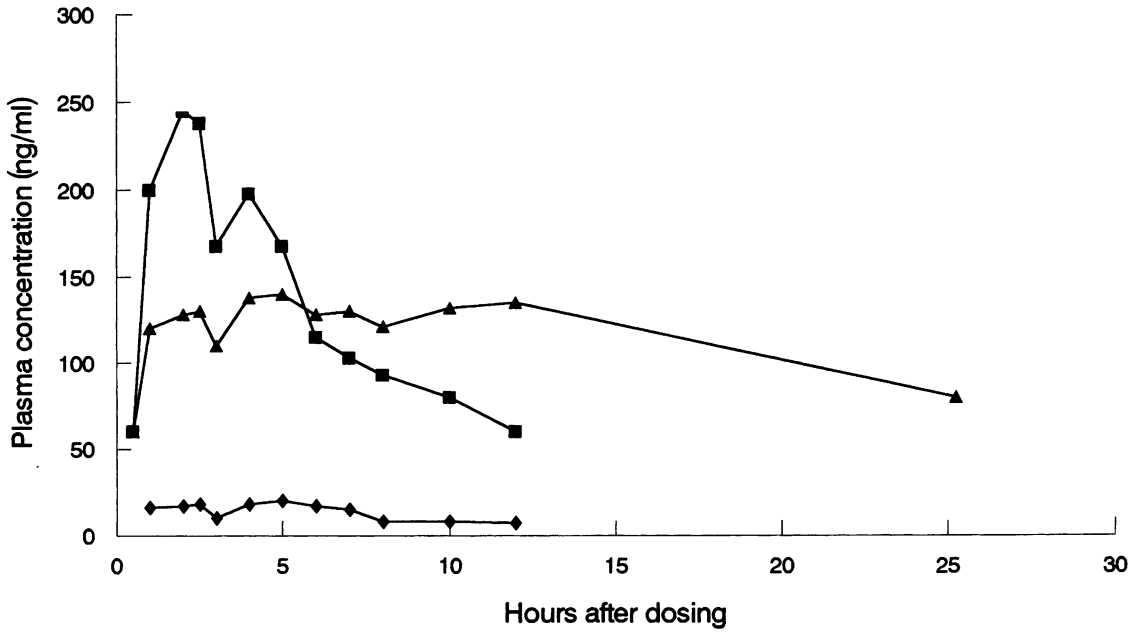

Figure 4 **Plasma levels of primaquine and its main metabolites in man after a single oral dose of 120 mg base** (adapted from Ref. 27)
■ Primaquine; ▲ COOH metabolite; ◆more polar metabolite

launched to discover novel alternatives for prophylaxis and treatment.

Unfortunately, primaquine has a short biological half-life in man (Figure 4). The US Army programme, therefore, included a search for novel 8-aminoquinolines and other tissue schizontocides that would be safer, longer acting, and perhaps have a wider spectrum of useful activity than that of primaquine[28]. From this search emerged a 5-phenoxy derivative identified first as WR 238,605 and now known as tafenoquine (see Figure 2). Chloroquine resistance and multidrug-resistance in the asexual blood stages of *P. falciparum* have since those days become a global problem.

Chloroquine-resistant *P. vivax*—a new challenge

A further difficulty has arisen with the emergence, in areas as far apart as the Southwest Pacific, Thailand, Somalia and Latin America, of strains of *P. vivax* whose asexual blood stages are solidly resistant to chloroquine. An initial warning in 1989[29] was followed by confirmatory reports from Papua New Guinea[30,31] and subsequently from Irian Jaya[32]. In a comparison of primaquine alone or in combination with chloroquine, using the standard doses employed for radical cure of vivax malaria, clinicians in Thailand[33] were surprised to find in 1994 that the intraerythrocytic infections responded nearly as well to primaquine as to the combination and warned that this inherent activity might well have been masking developing chloroquine resistance when the standard radical curative regimen was being employed. As part of a project to design a satisfactory clinical test to identify chloroquine-resistant *P. vivax*, Baird and his associates[34] decided to explore further the potential value of primaquine as a causal prophylactic against both *P. falciparum* and *P. vivax* in non-immune Javanese immigrants who had been resettled in highly endemic areas of Irian Jaya where, by then, both parasites were strongly resistant to chloroquine. Surprisingly, they found that a daily (adult) dose of 30 mg primaquine base given on alternate days after a meal for up to 19 consecutive weeks to individuals with normal G-6-PD was even better tolerated than weekly chloroquine at an adult dose of 300 mg base once weekly. Moreover, primaquine gave five times better protection than chloroquine against both parasites and especially against *P. vivax*. The trial was extended in 1995 in a further group of G-6-PD-normal volunteer settlers who took 30 mg primaquine base daily, 300 mg chloroquine base weekly or placebo for up to one year[35]. The excellent protective action of primaquine against both parasite species was confirmed, as was its safety, although transitory methaemoglobinaemia was present in those taking this compound. The prophylactic potential of primaquine against chloroquine-resistant *P. falciparum* was then compared with that of doxycycline, proguanil plus chloroquine, mefloquine or placebo in semi-immune children in a highly endemic area of western Kenya in an 11-week study[36]. Children with deficient G-6-PD or haemoglobin AS were excluded from the trial. Primaquine phosphate, 15 mg base, taken thrice weekly or once daily gave very good protection against falciparum parasitaemia and clinical malaria, as did 50 mg doxycycline daily or 125 mg mefloquine weekly. A regimen of 150 mg chloroquine base weekly plus 200 mg proguanil daily was significantly less protective but still much superior to placebo. Thus the potential scope for the use of primaquine appeared to be increasing (Table 4).

Still seeking the optimum way to pinpoint chloroquine resistance in *P. vivax*, Baird and his associates[37] decided to assess the therapeutic value of chloroquine with or without primaquine, in comparison with halofantrine, in their non-immune Javanese population in Irian Jaya. In a 28-day follow-up they observed that the therapeutic failure rate in patients receiving chloroquine with placebo as a single adult dose of 600 mg or 1.5 g over three days was eight times higher than that in others who received chloroquine with primaquine. The primaquine was administered either in daily doses of 30 mg base for 14 days, followed by the same dose on alternate days for a further 13 days, or in a later phase of the trial 60, 60 and 30 mg on each of three consecutive days. In the latter phase these regimens were compared with three six-hourly doses of halofantrine 500 mg base. Overall, the crude failure rate at 14 days was 44%, 5% and nil for chloroquine alone, chloroquine plus primaquine and halofantrine, respectively. At 28 days the corresponding figures were 79%, 15% and 6%. Baird and his colleagues concluded that these results were attributable in each group to the blood schizontocidal action of the various compounds. Moreover, it was their impression that there existed a synergistic action between chloroquine and primaquine against these largely

Table 4 **Potential future uses for primaquine or other 8-aminoquinolines**

***Species of* Plasmodium**	***Indication***
P. falciparum, P. vivax (and ? others)	Causal prophylaxis
Chloroquine-resistant *P. falciparum* and *P. vivax*	Blood schizontocide in combination with chloroquine, halofantrine, etc.
Chloroquine-resistant *P. vivax*	Radical curative in combination with chloroquine, halofantrine, etc.

chloroquine-resistant parasites. From correspondence with Kevin Baird, it transpired that these clinical observations by chance coincided with experiments that we were conducting in chloroquine-resistant rodent malaria with the new 8-aminoquinoline, WR 238,605.

We therefore added to our protocol a combination of chloroquine with primaquine to compare with data we had already obtained with chloroquine and WR 238,605 (Figure 5). With both combinations we observed clearcut synergism (or drug resistance reversal) against chloroquine-resistant *P. yoelii* ssp. NS[38], thus giving extra weight to Baird's hypothesis that chloroquine and primaquine were synergistic against chloroquine-resistant *P. vivax* and the earlier suggestion of the Thai investigators that this combination might mask hitherto undetected chloroquine resistance in *P. vivax*.

TAFENOQUINE—THE NEW HOPE

Thus the range of potential applications for primaquine has been extended from those of anti-relapse and gametocytocidal use to causal prophylaxis and deployment as a blood schizontocide. Nevertheless, it still has to be borne in mind that the blood schizontocidal action of this compound against *P. falciparum*, and especially multidrug-resistant strains of that species, is very poor, whilst against *P. vivax* it is still not as effective a blood schizontocide as, for example, halofantrine. The development of tafenoquine from its progenitors, quinine, methylene blue and pamaquine, has been achieved at a good moment.

When tafenoquine was first investigated in the US Army programme it was as a substitute for primaquine that would be more effective as a radical curative drug against relapsing vivax malaria but in this role the development work did not receive a high priority. However, once its potent blood schizontocidal activity against multidrug-resistant asexual blood stages of *P. falciparum* was identified, its preclinical development moved into high gear.

Orally administered tafenoquine is slowly absorbed and, in marked contrast to primaquine, slowly metabolized, with an elimination half-life of 14 days[39]. Its possible haemolytic action relative to primaquine in G-6-PD-deficient individuals (a generic hazard of 8-aminoquinolines) is currently being investigated but the agent is otherwise better tolerated than primaquine. A preliminary study in non-immune volunteers showed that a single dose of 600 mg tafenoquine, administered the day before challenge with *P. falciparum*-infected mosquitoes, fully protected three of four subjects and significantly impeded the onset and severity of parasitaemia in a fourth[40]. An extended study in semi-immune volunteers in Kenya confirmed that a single dose of 250 or 500 mg tafenoquine given once weekly for 13 weeks provided a high level of protection against *P. falciparum* in a holoendemic area[41].

In sporozoite-infected rhesus monkeys, tafenoquine had a causal prophylactic action against *P. cynomolgi*[42], thus confirming our observations with the rodent model[38]. Tafenoquine cured established asexual parasitaemia with the primaquine-tolerant Chesson strain or the chloroquine-resistant AMRU 1 strain of *P. vivax* in *Aotus* monkeys[43]. In combination with chloroquine it had an additive effect against blood-induced infection with the AMRU 1 strain in *Aotus*[44] (in contrast to the synergistic action observed against chloroquine-resistant *P. yoelii* ssp. NS in mice[38]). Tafenoquine eliminated the hypnozoite stage of relapsing *P. cynomolgi* in rhesus monkeys (the standard model for

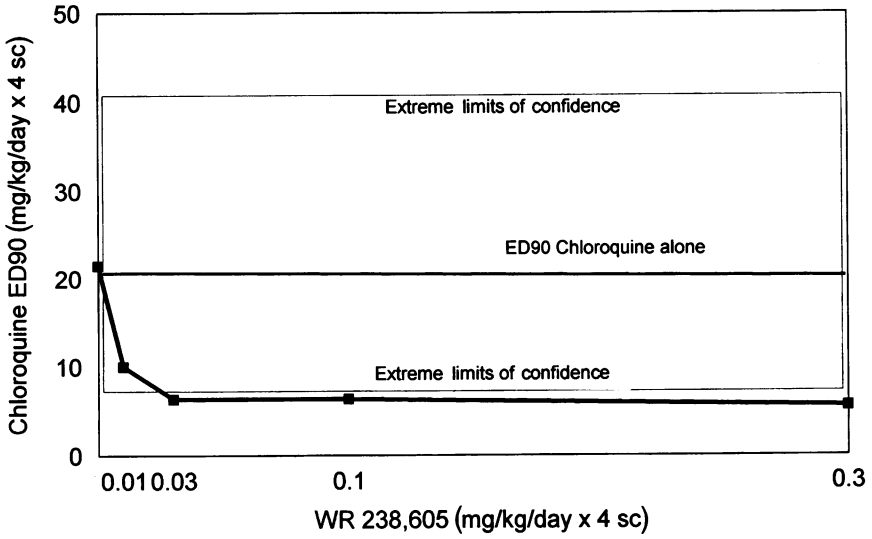

Figure 5 **Synergy between WR 238,605 (tafenoquine) and chloroquine against *P. yoelii* ssp. NS in '4-day test' of blood schizontocidal activity** (adapted from Ref. 38). Chloroquine alone ED_{90}=19.5, WR 238,605 alone=3.4 mg/kg.day × 4 sc

radical curative drugs against *P. vivax*)[43] and a single dose of 500 mg has produced radical cure of naturally acquired vivax infection in patients in Thailand[45]. Moreover, a gametocytocidal action of tafenoquine in rodent malaria models has been reported in chloroquine-sensitive *P. berghei* ANKA[19] and in chloroquine-resistant *P. yoelii* ssp. NS in mice[38].

These data indicate that tafenoquine has promise as a novel causal prophylactic, possibly in a single weekly dose, against *P. falciparum* and as a radical curative agent against *P. vivax*. Its potent blood schizontocidal activity against both these parasites, confirming that first reported in rodent and simian malaria models, also suggests that tafenoquine may have a place in the treatment of established infection with multidrug-resistant *P. falciparum* or chloroquine-resistant *P. vivax*. In addition, the gametocytocidal action of tafenoquine may contribute to a reduction of transmission if the compound is eventually used widely under field conditions. In whatever indication it may come to be deployed it will be essential to avoid administering tafenoquine to any individual who has a significant deficiency of G-6PD status and it is to be hoped that a simple, cheap and easily used test to determine G-6PD status will soon become available. A further word of caution is needed. Blood stage parasites readily become resistant, sooner or later, to any antimalarial that is used on its own. This applies to primaquine and undoubtedly will also apply to tafenoquine. Should it fulfil its promise in further clinical trials, the potential protective role of carefully selected drug combinations with tafenoquine must be considered mandatory if this compound is to be deployed in endemic areas. The artemisinins, with their rapid onset of action[46], suggest themselves as appropriate partners. On existing evidence, tafenoquine could be a major new antimalarial for the new millennium.

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