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# *In-vitro* activity of primaquine against the asexual blood stages of *Plasmodium falciparum*

Primaquine, an 8-aminoquinoline, has been shown to inhibit the development of the hepatic stages (hypnozoites and schizonts), gametocytes, and asexual, intra-erythrocytic forms of malarial parasites (Peters and Robinson, 1987). This triple action is unique among antimalarial drugs. Primaquine is widely used for its anti-hypnozoite action (i.e. to prevent relapses of *Plasmodium vivax* or *P. ovale* infection) and interest in its use as a causal prophylactic, to eliminate the liver schizonts of all four *Plasmodium* species infecting man, before they mature to invade the erythrocytes, has increased in recent years (Fryauff *et al.*, 1995). Primaquine may also be prescribed as a gametocide to patients in areas with low levels of transmission.

The blood schizonticidal action of primaquine was initially studied in six healthy volunteers who were inoculated with a chloroquine-sensitive strain of *P. falciparum* (P-F-6/Panama) before treatment with the drug at doses of 30 or 45mg/day for 14 days (Arnold *et al.*, 1955). Only one of the volunteers cleared asexual parasitaemia within the first 3 days of treatment but recrudescence occurred on day 23. The other five volunteers failed to clear asexual parasitaemia and required chloroquine before the end of the 14-day course of primaquine. The results of a recent re-evaluation of its blood schizonticidal effect have shown that primaquine, in standard therapeutic doses with or without chloroquine, is effective in clearing *P. vivax* parasitaemias (Pukrittayakamee *et al.*, 1994; Baird *et al.*, 1995). In the present study, the potential of primaquine as a blood schizonticide against *P. falciparum* was assessed and compared, *in vitro*, with that of other blood schizonticidal drugs.

Twenty–eight, fresh, clinical isolates of *P. falciparum* were obtained, before treatment, from symptomatic, indigenous patients in Yaoundé, Cameroon, in 1997. The isolates selected came from patients who only had *P. falciparum* infection, had $>0.2\%$ of their erythrocytes infected, and gave a negative result when their urine was tested by the Saker–Solomons test. If the parasitaemia was $>1.0\%$, uninfected erythrocytes were added to adjust the parasite density to 0.5%. The study was approved by the Cameroonian National Ethics Committee.

Primaquine diphosphate (Sigma) was dissolved in and diluted with sterile distilled water to give doubling, final dilutions between 0.5 and 32 $\mu$M. Each concentration was distributed in triplicate in 96-well, tissue-culture plates. Solutions of other test compounds were prepared as described previously (Ringwald *et al.*, 1996). The microtest developed by Desjardins *et al.* (1979) was then used to determine the blood schizonticidal activity of each compound over a 48-h incubation at 37°C in 5% $CO_2$. [G-$^3$H]Hypoxanthine (1 $\mu$Ci/well) was added after 18 h of exposure to the drug, and its subsequent incorporation into the DNA of the parasites was measured in a liquid-scintillation counter (Wallac 1410; Pharmacia). Parasite growth was plotted against drug concentration, and a sigmoid curve was fitted and analysed by non-linear regression using Prism™ software (GraphPad Software, San Diego, CA). The in-vitro schizonticidal activity of each compound was then expressed as the concentration ($IC_{50}$) halving the parasite growth (i.e. incorporation of radiolabel) seen in drug-free, control wells.

The geometric mean $IC_{50}$ of primaquine was 1.44 $\mu$M [95% confidence intervals (CI) = 1.27–1.64 $\mu$M; range = 0.76–2.46 $\mu$M; see Fig.].

By comparing the geometric mean value (and CI) for the $IC_{50}$ of primaquine with that of the major blood schizonticides used in Africa (see Fig.), it can be seen that pri-

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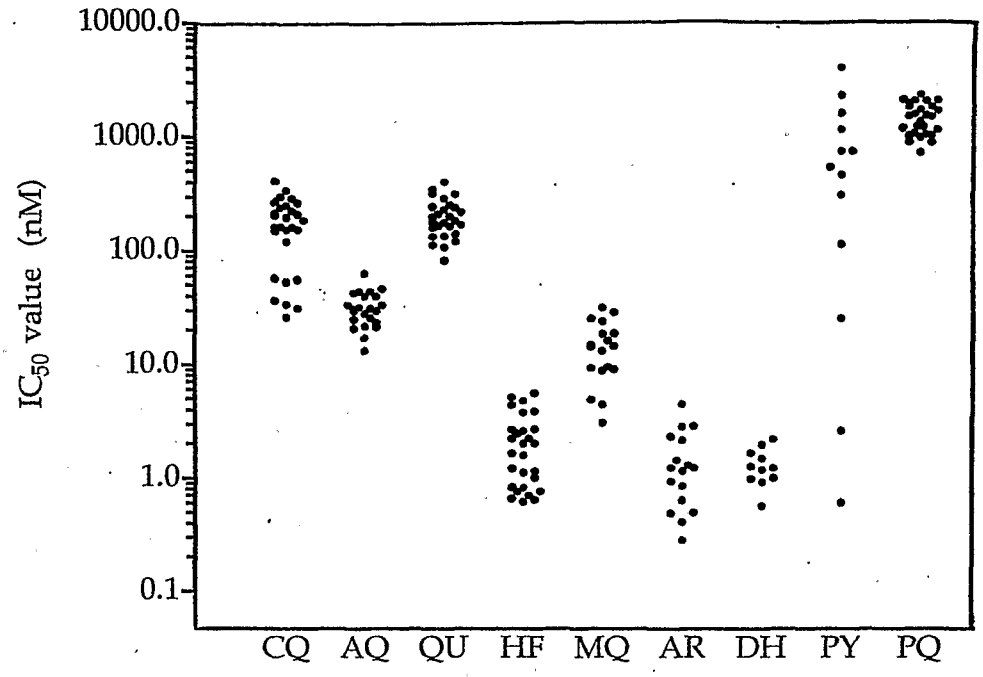


Fig. Distribution of the median inhibitory concentrations ($IC_{50}$) for chloroquine (CQ), monodesethylamodiaquine (AQ), quinine (QU), halofantrine (HF), mefloquine (MQ), artesunate (AR), dihydroartemisinin (DH), pyrimethamine (PY), and primaquine (PQ) against the asexual blood stages of Cameroonian isolates of *Plasmodium falciparum*.

maquine is 7.4-, 10-, and 47-fold less active than quinine [195 (167–227) nM], chloroquine [141 (104–192) nM], and monodesethylamodiaquine [30.8 (26.4–35.9) nM], respectively. The synthetic aminoalcohols—mefloquine [13.0 (9.4–18.0) nM] and halofantrine [1.74 (1.32–2.30) nM]—and artemisinin derivatives—artesunate [1.16 (0.78–1.73) nM] and dihydroartemisinin [1.27 (0.98–1.64) nM]—are approximately 100–1000 times more active than primaquine. Some of the isolates, which were pyrimethamine-resistant, gave similar $IC_{50}$ for pyrimethamine and primaquine but the others had lower $IC_{50}$ for pyrimethamine (see Fig.).

Bhasin and Trager (1987), using the HB3/Honduras clone of *P. falciparum*, found that 1 μg primaquine/ml (i.e. 2.1 μM) was required to inhibit approximately 50% of asexual parasite growth *in vitro*. Although this $IC_{50}$ value falls within the range found in the present study, several methodological differences (parasite growth assessed by microscopical examination, exposure for 96 h and use of only three drug concentrations in the earlier study) preclude direct comparison of the results of the two investigations. In another study, using six, culture-adapted strains of *P. falciparum* and the same drug assay as in the present study (except for the higher initial parasitaemias of 1%–2%), primaquine was found to be more potent against the chloroquine-resistant strains ($IC_{50} = 0.59$–$0.71$ μM) than against the chloroquine-sensitive ($IC_{50} = 5.5$–$14$ μM) (Geary *et al.*, 1987). Although these $IC_{50}$ are similar to those in the present study, none of the present isolates exhibited $IC_{50}$ of $> 2.5$ μM. However, as in the study of Geary *et al.* (1987), the geometric mean $IC_{50}$ (and CI) of the present chloroquine-resistant isolates [1.30 (1.14–1.48) μM] were significantly lower than those of the chloroquine-sensitive isolates

[1.99 (1.70–2.33) $\mu$M; $P < 0.05$ by unpaired $t$-test].

Although primaquine is principally metabolised into a carboxylic acid derivative in man (Breckenridge *et al.*, 1987), the full and complex process of its human metabolism, which yields numerous metabolic products, has not been fully elucidated. Some of the metabolites exhibit higher activities against the hepatic stages of malarial parasites than primaquine itself and may be associated with drug-induced haemolysis in patients with glucose-6-phosphate-dehydrogenase deficiency (Strother *et al.*, 1981; Baird *et al.*, 1986; Bates *et al.*, 1990). The blood schizonticidal activities of the metabolites were not determined in the present study. In the *P. berghei*–rodent model, primaquine itself has been found to possess a high activity against the asexual intra-erythrocytic parasites, whereas the carboxylic acid metabolite appears to be essentially devoid of blood schizonticidal action (Peters and Robinson, 1987). Further studies are needed to define the various roles of primaquine metabolites in man.

In conclusion, primaquine is less active *in vitro* than the standard blood schizonticides (chloroquine, amodiaquine, pyronaridine, quinine, mefloquine, halofantrine, artemisinin derivatives, atovaquone and pyrimethamine), which have $IC_{50}$, against the blood schizonts of drug-sensitive isolates, of $< 500$ nM (quinine) or even $< 100$ nM (others) (Ringwald *et al.*, 1996). However, the blood schizonticidal activity of primaquine *in vitro* is superior to that of the antibiotics used in antimalarial chemotherapy, such as doxycycline and clindamycin (Divo *et al.*, 1985; Basco and Le Bras, 1993). Although the results of in-vitro studies should not be extrapolated to in-vivo conditions, and clinical studies are needed to confirm the present results, the moderate blood schizonticidal activity observed here may indicate that primaquine alone may not be curative against *P. falciparum* infections. However, drug combinations exhibiting synergistic effects with primaquine may render this 8-aminoquinoline useful against the asexual blood stages of *P. falciparum*.

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