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In vitro activity of chloroquine, the two enantiomers of chloroquine, desethylchloroquine and pyronaridine against *Plasmodium falciparum*

S. FU[1], A. BJÖRKMAN[2], BIRGITTA WÅHLIN[3], D. OFORI-ADJEI[1], Ö. ERICSSON[1] & F. SJÖQVIST[1]
[1]Department of Clinical Pharmacology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden, [2]Department of Infectious Diseases, Karolinska Institute, Roslagstull Hospital, S-114 89 Stockholm, Sweden, and [3]Department of Immunology, University of Stockholm, S-106 91 Stockholm, Sweden

A 48 h *in vitro* test was conducted to compare the susceptibility of two strains of *Plasmodium falciparum* to chloroquine, the two enantiomers of chloroquine, desethylchloroquine and the new antimalarial drug pyronaridine. The five compounds similarly inhibited the chloroquine sensitive strain. However, desethychloroquine was less active and pyronaridine was much more active than chloroquine and its enantiomers against the chloroquine resistant strain.

Keywords *Plasmodium falciparum* *in vitro* test racemic chloroquine enantiomers of chloroquine desethylchloroquine pyronaridine

Introduction

Plasmodium falciparum malaria has become increasingly resistant to chloroquine (CQ) in different parts of the world (Bruce-Chwatt, 1982). Hence, full knowledge of the efficacy of different derivatives of CQ and of alternative compounds is essential. The technique for continuous culture of *P. falciparum in vitro* (Trager & Jensen, 1976) has provided the possibility of reproducible assessment of the susceptibility of *P. falciparum* to different compounds (Nguyen-Dinh & Trager, 1980).

Commercially available CQ is a racemic mixture of two enantiomers. The antimalarial activity of the two enantiomers has been studied in mice, and it was reported that (+)−CQ is more active than the other isomer (Fink *et al.*, 1979; Haberkorn *et al.*, 1979). The toxicity (LD_{50}) was lower for the (+) enantiomer in the mouse model (Haberkorn *et al.*, 1979). However, the metabolism of CQ may be stereoselective (Titus *et al.*, 1948), and an *in vivo* comparison of the activity is therefore difficult to interpret.

Desethylchloroquine (DCQ), the main metabolite of CQ, appears in appreciable concentrations in the blood after administration of CQ (Gustafsson *et al.*, 1983). *In vitro*, CQ and DCQ were equally active against sensitive *P. falciparum* isolates but for resistant isolates, the metabolite was significantly less active than the parent compound (Aderounmu, 1984; Verdier *et al.*, 1984).

Pyronaridine (PY, 7-chloro-2-methoxy-10-[3,5-bis(pyrrolidinomethyl)-4-hydroxyanilino] benzo-[b]-1,5-naphthyridine) is a new antimalarial agent, which has been synthesized in China (Chen, 1981). It is related to certain other antimalarials, e.g. mepacrine, which has a similar ring system. Tests with *P. berghei* in mice have shown that PY has higher schizonticidal activity than CQ (Institute of Parasitic Diseases, 1980). Toxicological studies have shown a potential embryotoxicity in rats with high doses (Ni *et al.*, 1982) but lower toxicity than CQ when given orally to mice, rats, dogs and monkeys (Institute of Parasitic Diseases, 1980). The cumulative

Correspondence: Dr Ö. Ericsson, Department of Clinical Pharmacology, Huddinge University Hospital, S-141 86 Huddinge, Sweden

lethal dose was much higher than that of CQ (Institute of Parasitic Diseases, 1980). Furthermore, clinical trials have indicated high efficacy against CQ resistant malaria and few disturbing side-effects (Xu *et al.*, 1982a,b; Lapierre, 1982).

The aim of the present investigation was to assess the *in vitro* activity of CQ, (+)−CQ, (−)−CQ, DCQ and PY against two strains of *P. falciparum*.

Methods

Parasites

Two *P. falciparum* strains, adapted to continuous culture by the candle jar method (Trager & Jensen, 1976) were used in this study. Strain F32 from Tanzania, isolated in 1978 was known to be sensitive to CQ and strain KI from Thailand, isolated in 1979 was known to be resistant to CQ.

Test compounds

Racemic CQ phosphate was obtained from Kabi-Vitrum AB, Stockholm, Sweden. (+)−CQ and (−)−CQ were synthesised as described by Blaschke *et al.* (1978). DCQ was a gift from Sterling-Winthrop AB, Solna, Sweden and PY phosphate was synthesised at the Institute of Parasitic Diseases, Shanghai, China.

In vitro *growth inhibition assay*

The culture medium consisted of RPMI 1640 (GIPCO Grand Island, NY, USA), supplemented with Hepes buffer (20 mM), $NaHCO_3$ (0.2%), human serum (15%), glutamine (2 mM) and gentamicin (25 μg ml^{-1}).

For the F32 strain, the different drug concentrations in the medium were 6.25×10^{-10} M to 2×10^{-8} M of each compound respectively, and for the KI strain, the drug concentrations were 6.25×10^{-9} M to 2×10^{-7} M of CQ (racemic, (+) or (−)), 6.25×10^{-9} M to 4×10^{-7} M of DCQ and 2×10^{-10} M to 2×10^{-7} M of PY.

In some experiments, h.p.l.c. analysis of the CQ derivatives in the medium was made at the end of the *in vitro* assays to ascertain adequate drug concentrations.

Growth inhibition of *P. falciparum* by the test compounds was measured by a modified 48 h test (Wåhlin *et al.*, 1984). In brief, the starting non-synchronized culture had a parasitemia of 0.12–0.77% and a haematocrit of 2%. Quadruplicate aliquots of 100 μl culture were incubated with 100 μl culture medium or test compound at various concentrations in 96 well flat-bottomed microculture plates for 48 h at 37° C by the candle jar method. After incubation, the erythrocytes (RBC) from each well were separately washed twice with Tris-buffered Hank's solution (TH), pH 7.2, and diluted to 1% in TH. Monolayers of infected RBC were formed in the wells of eight well multitest slides coated with 0.06 M bicarbonate buffer, pH 9.6. Each test sample was set up in duplicate. The monolayers were fixed by two treatments for 10 s with 1% glutaraldehyde. The slides were washed with distilled water and air dried. To visualize the parasites, the monolayers were stained with acridine orange (10 μg ml^{-1}) for 10 s washed with distilled water and counted using an UV-microscope. Twenty-five microscope fields (200 RBC/field) were screened for each well. The percentages of parasitemia given are the mean values from 40,000 RBC counted.

If the multiplication rate in the control wells was below twofold, the test was discarded. Each strain was tested three times for the five compounds. The results of the three tests were added. In the statistical evaluation of the different regression lines of growth inhibition, analysis of variants was used.

Results

There was little variation between the three replicate tests and the results are summarized in Figures 1 and 2. The two CQ enantiomers and and the racemate were equally active against the sensitive *P. falciparum* strain, but (−)−CQ was slightly less active than (+)−CQ and the racemate against the resistant strain ($P < 0.05$). DCQ was equally active against the sensitive strain but significantly less active than the parent compound against the resistant strain ($P < 0.05$). PY was highly active against both strains, with an even higher degree of efficacy against the strain which was resistant to CQ ($P < 0.05$).

In order to ascertain that chloroquine was not adsorbed to the plastic microculture plates the concentration was checked by h.p.l.c. analysis. No adsorption losses were noticed.

Discussion

The aim of the present study was to evaluate and compare the antimalarial activity of a series of compounds. To eliminate the effect of differential disposition, which may complicate the evaluation of an *in vivo* test, we preferred to use an *in vitro* technique.

There was only one minor difference in the potency of racemic CQ and the two enantiomers,

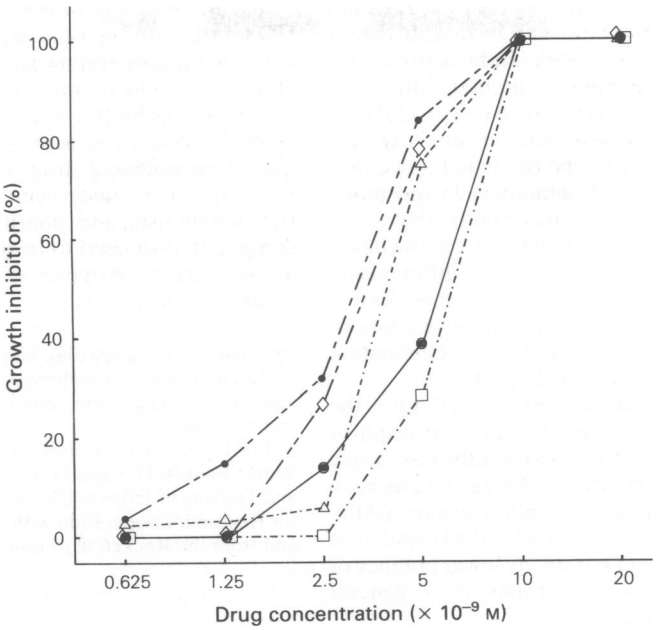

Figure 1 *In vitro* response of the F32 strain of *P. falciparum* to different concentrations of racemic CQ (◇——◇), (+)−CQ (●——●), (−)−CQ (△·····△), DCQ (□·····□) and PY (◉——◉).

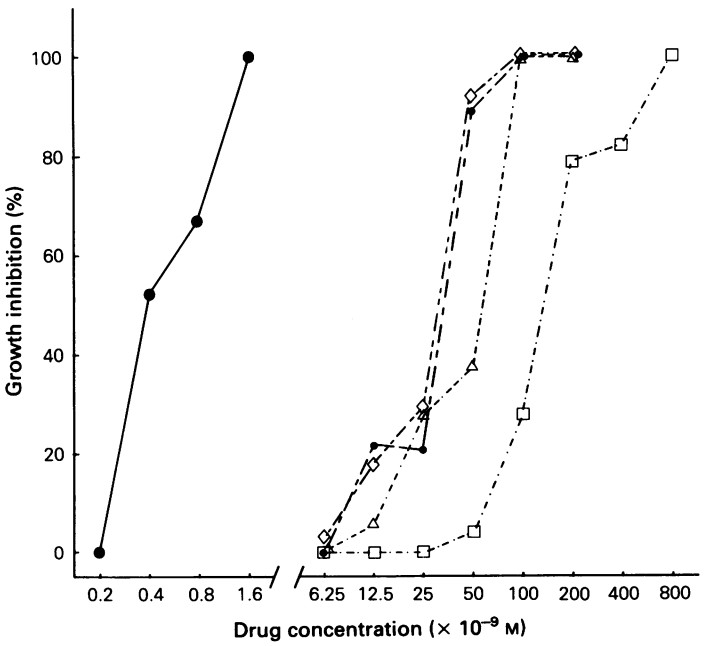

Figure 2 *In vitro* response of the KI strain of *P. falciparum* to different concentrations of racemic CQ (◇——◇), (+)−CQ (●——●), (−)−CQ (△·····△), DCQ (□·····□) and PY (◉——◉).

(−)−CQ being less active against the resistant strain. This finding is at variance with earlier reports (Fink *et al.*, 1979; Haberkorn *et al.*, 1979), which have shown a greater difference, (+)−CQ being more active than (−)−CQ. However, these studies were made using *P. vinckei* and *P. berghei* in mice, and the actual concentrations of CQ and its enantiomers in the blood were not determined. It may well be that (+)−CQ and (−)−CQ are eliminated from the blood at different rates, and the apparent difference in activity may then only be due to stereoselective disposition. In fact our preliminary investigations suggest that there is stereoselective elimination of CQ in man (unpublished data).

DCQ showed roughly the same activity as the parent drug against the CQ sensitive strain of *P. falciparum* but was significantly less active against the resistant strain. Similar results have recently been reported by other groups (Aderounmu, 1984; Verdier *et al.*, 1984) and it is possible that the comparatively lower potency of DCQ against resistant strains is a general phenomenon.

The high activity of PY *in vitro* against both CQ sensitive and resistant strains is in agreement with findings in clinical trials in China (Xu *et al.*, 1982a,b). Hence, with the apparently low degree of toxicity (Institute of Parasitic Diseases, 1980; Xu *et al.*, 1982a,b) PY may represent an important potential drug for the treatment of CQ resistant *P. falciparum* malaria. However, the metabolism and pharmacokinetics of the compound also need to be investigated, as well as the intrinsic activities of major metabolites against *P. falciparum*.

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