In vitro activity of the enantiomers of mefloquine, halofantrine and enpiroline against *Plasmodium falciparum*

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The *in vitro* activity of the enantiomers of mefloquine, halofantrine and enpiroline was compared against chloroquine-resistant and -susceptible strains of *Plasmodium falciparum* using a semi-micro drug susceptibility test. For each strain, the corresponding enantiomers exhibited similar activities. The enantiomers of halofantrine were the most active against both susceptible and resistant strains, followed by the enantiomers of mefloquine and enpiroline.

Keywords *Plasmodium falciparum* malaria mefloquine halofantrine enpiroline enantiomers

Introduction

The spread of chloroquine-resistant *Plasmodium falciparum* has been reported from most regions of the world where malaria is endemic (WHO, 1989). New blood schizontocides effective against resistant malaria parasites include mefloquine, halofantrine and enpiroline, all of which are aminoalcohols chemically related to quinine.

Mefloquine, a 4-quinolinemethanol, has two asymmetric carbon atoms and is used clinically as a 50:50 racemic mixture of the *erythro* isomers (dextrorotatory 11R,2'S and laevorotatory 11S,2'R) (Figure 1). Halofantrine, a 9-phenanthrenemethanol, has one asymmetric carbon atom and is used clinically as a racemate. Enpiroline, a 4-pyridinemethanol, was shown to be highly active against multidrug-resistant strains of *P. falciparum*, both *in vitro* (Desjardins *et al.*, 1979) and in human volunteers, with few side-effects (Cosgriff *et al.*, 1984). It has two asymmetric carbon atoms and is used as a racemic mixture of the *threo* epimers. The chemical structures of the enantiomers of the three drugs are shown in Figure 1. All clinical studies on mefloquine, halofantrine and enpiroline have been carried out with their racemic mixtures.

After oral administration, the plasma concentrations of (–)-mefloquine and (+)-halofantrine are significantly higher than those of their respective enantiomers (Gimenez *et al.*, 1990, 1992). Since there may also be a stereoselective difference in their pharmacological action, as in the case of chloroquine (Fu *et al.*, 1986), we compared the *in vitro* activities of the enantiomers of mefloquine, halofantrine and enpiroline against chloroquine-resistant and -susceptible strains of *P. falciparum*.

Methods

Parasites

Two strains of *P. falciparum* were maintained in a continuous culture using the method of Trager & Jensen (1976). The chloroquine- and quinine-resistant strain, FCM 29 (IC₅₀ of chloroquine = 1670 nmol l⁻¹), was isolated from a traveller returning from the Cameroon and was cloned by a limiting dilution method. The chloroquine- and quinine-susceptible strain, L-3 (IC₅₀ of chloroquine = 34.9 nmol l⁻¹), was obtained from a patient returning from Ivory Coast.

Drugs

Racemic mefloquine hydrochloride was obtained from Hoffmann-La Roche, Basel, Switzerland. Racemic halofantrine hydrochloride was obtained from Smith Kline Beecham, Hertfordshire, UK. The purity of racemic mefloquine and halofantrine and their isomers was checked by IR and MNR spectrometry. The enantiomers of mefloquine and halofantrine were separated and purified by fractional crystallization (Carroll & Blackwell, 1974; Carroll *et al.*, 1978). The enantiomers were analysed by chiral h.p.l.c. (Gimenez *et al.*, 1990) to ascertain their isomeric purity (98.1–98.9%). Optically pure (+) enpiroline phosphate (WR 247,734) and (–) enpiroline phosphate (WR 247,733) were kindly provided by Dr D. E. Kyle of the Walter Reed Army Institute of Research. Stock solutions were prepared in methanol. Twofold dilutions (final concentrations 2.5–400 nmol l⁻¹ for mefloquine hydrochloride; 0.25–32 nmol l⁻¹ for halofantrine hydrochloride; 12.5 to 1600 nmol l⁻¹ for

Figure 1 Chemical structures of the enantiomers of mefloquine, halofantrine and enpiroline. Mefloquine: (±)-erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol. Halofantrine: (±)-1,3-dichloro-α-[2(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol. Enpiroline: (±)-threo-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridinemethanol.

enpiroline phosphate) were distributed in 24-well plates in triplicate.

Stereoselective h.p.l.c. assay

Stereoselective analysis of mefloquine enantiomers was performed using a Pirkle type (S)-naphthylurea column (S.F.C.C./Shandon, Eragny, France) with a mobile phase composed of a mixture of hexane/2-propanol/methanol (82/4/14, v/v/v) running at 1.0 ml min⁻¹ and at ambient temperature (Gimenez *et al.*, 1990). Detection was by ultraviolet spectrophotometry at 285 nm.

Stereoselective analysis of halofantrine enantiomers was performed using a protein ovomucoid Ultron ES-OVM chiral column (S.F.C.C./Shandon, Eragny, France) with a mobile phase composed of sodium phosphate buffer 0.01 M pH 5.0/acetonitrile (75/25, v/v) running at 1.0 ml min⁻¹ and at ambient temperature (Gimenez *et al.*, 1992). Detection was made by spectrofluorimetry with excitation and emission wavelengths of 260 nm and 380 nm, respectively.

In vitro drug susceptibility test

The semi-micro method of Le Bras & Deloron (1983) was used. Briefly, a suspension (700 µl/well) of parasitized erythrocytes in RPMI 1640 medium (2.5% v/v haematocrit, 0.1–1.0% v/v initial parasitaemia), supplemented with 10% v/v human serum, 25 mM of HEPES, and 25 mM of NaHCO₃, was distributed in the predosed plates. The plates were incubated for 42 h at 37° C in 5% O₂, 5% CO₂, and 90% N₂. [G-³H]hypoxanthine (specific activity 4.3 Ci mmol⁻¹) was added 18 h later to assess parasite growth. The contents of each well were collected and washed using a cell harvester. The amount of radioactivity incorporated by the parasites was measured by liquid scintillation counting (Wallac 1410, Pharmacia, Sweden). The estimation of 50% inhibitory concentrations (IC₅₀) was done by linear regression analysis of log-dose-response curves. Analysis of variance

and covariance was used to compare the linear regression lines, with the significance level set at 95%. The experiments were replicated three to five times.

Results

The enantiomers tested in this study were effective against the chloroquine-susceptible and -resistant strains of *P. falciparum*, having IC₅₀ values below 56 nmol l⁻¹. There were no significant differences (*P* > 0.05) in the IC₅₀ values of the individual enantiomers of each pair (Figure 2). For both parasite strains, the order of activity was halofantrine > mefloquine > enpiroline.

Chiral h.p.l.c. analysis of the inoculum after 42 h of incubation confirmed the presence of the enantiomers of mefloquine and halofantrine in the plates, with no enantiomeric inversion.

Discussion

A study in mice has shown that the four isomers of mefloquine are equally active against *P. berghei* (Carroll & Blackwell, 1974). However, this was not confirmed in another study which showed that the *erythro* isomers were twice as effective as the *threo* isomers against *P. berghei* in mice. Nevertheless, all four isomers were equally effective against *P. falciparum* in the owl monkey *Aotus trivirgatus* (Schmidt *et al.*, 1978). The enantiomers of halofantrine are equally active against *P. berghei* in mice (Carroll *et al.*, 1978). The results of the present study indicate that the enantiomers of mefloquine and halofantrine are equally active *in vitro*. Thus it appears that despite wide phylogenetic and biochemical differences between *P. berghei* and *P. falciparum*, our *in vitro* results are in agreement with previous *in vivo* findings.

There are no published data on the activity of the

Figure 2 *In vitro* activity of the enantiomers of mefloquine (MQ), halofantrine (HF) and enpiroline (EN) against a) the chloroquine-resistant strain FCM 29 and b) the chloroquine-susceptible strain L-3 of *Plasmodium falciparum*. Each point represents the mean of three to five replicate tests.

enantiomers of enpiroline. However, based on the finding that the crystal structure of the enantiomers of enpiroline can be superimposed on either of the two diastereoisomers quinine and quinidine, Karle & Karle (1989) suggested that both enantiomers of enpiroline may be active against malaria parasites. The results of the present study confirm this prediction. We have found mean ($\pm$ s.d.) IC_{50} values of quinine and quinidine of 1120 ± 228 and 515 ± 142 nmol l⁻¹ for strain FCM 29, and 178 ± 26 and 55 ± 10 nmol l⁻¹ for strain L-3,

respectively. This two to threefold difference in their activity is consistent with the findings of others (Wesche & Black, 1990).

In contrast to quinine and quinidine, mefloquine, halofantrine and enpiroline do not exhibit any stereoselective action against *P. falciparum* *in vitro*. However, stereoselective differences in the pharmacokinetics of mefloquine and halofantrine (Gimenez *et al.*, 1990, 1992) suggest that (-)-mefloquine and (+)-halofantrine are more active than their antipodes *in vivo*.

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