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In Vitro Activity of Mefloquine and its Enantiomers Against Plasmodium falciparum

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ABSTRACT

The in vitro activity of rac-mefloquine hydrochloride and its pure enantiomers was tested against a chloroquine-resistant (PF.IBS2) strain of *Plasmodium falciparum*. The parasite isolated from Iranian patients was cultured in vitro by the candle jar method described by Tranger and Jensen and was exposed to the racemic mefloquine or its enantiomers over the concentration range of 10^{-9} to 10^{-4} M. Neither rac-mefloquine nor the enantiomers showed antiparasitic activity at 10^{-9} M. The (+)-mefloquine was more potent than the (-)-mefloquine and the racemate by IC_{50} equal to 1.17 µM in comparison to 4.09 µM.

Keywords: *mefloquine, enantiomer, antiparasite, Plasmodium falciparum*

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Chiral drugs are currently used as racemates. It has been demonstrated that in most cases two enantiomers of a chiral drug have different pharmacological activities. Mefloquine (MFQ), rac-erythro-α-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol, with two asymmetric carbon atoms has been used as a racemic 50:50 mixture in prophylaxis and treatment of the resistant strains of *Plasmodium falciparum* [1]. Various studies demonstrated that MFQ pharmacokinetics is highly stereoselective. The peak concentration and the area under the curve (AUC) of the (-)-MFQ have been significantly higher than those of the (+)-MFQ in blood and plasma after oral administration [2-4]. Higher concentration of the (+)-MFQ in rat plasma after oral administration has also been reported [5].

There are conflicting reports about the antimalarial activity of MFQ enantiomers. No significant difference is observed between antimalarial activities of enantiomers against *Plasmodium berghei* or *Plasmodium yoelli* in rodents [6, 7]. In vitro activity of MFQ enantiomers on two chloroquine-resistant and susceptible strains of *Plasmodium falciparum* showed similar activities for both enantiomers [8]. In another report [9], the (+)-enantiomer of MFQ has shown 1.69-1.81 times more activity than the (-)-MFQ against chloroquine-sensitive (Sierra Leone D-6) and chloroquine-resistant (Indochina W-2) strains of *Plasmodium falciparum* in vitro.

Conflicting reports on the antimalarial activity of MFQ enantiomers encouraged us to perform a new study on the activity of these enantiomers on a *Palsmodium falciparum* strain PF.IBS2 isolated from Iranian patients in southern parts of Iran.

MATERIALS AND METHODS

Chemicals and Reagents. Racemic MFQ.HCl was purchased from Roche (Basel, Switzerland). The (-)- and (+)-MFQ enantiomers were resolved with (+)-3-bromo-8-camphorsulphonic acid ammonium salt (Aldrich, Milwaukee, WI, USA) according to Carrol & Blackwell [6]. The enantiomeric purity of the resolved enantiomers was established using a modified indirect HPLC method of Bergqvist et al [10]. The derivatization was performed using (+)-1-(9-fluorenyl) ethyl chloroformate (FLEC) (18 mM in acetone) from Fluka (Buchs, Switzerland) and separation of the diastereomers was performed using a Novapak C18 cartridge column (150×3.9 mm, 4 µm Waters, Milford, MA). The mobile phase consisted of acetonitrile-water-acetic acid (730:270:0.7, v/v) at a flow rate of 1 ml min^{-1}. Detection was performed using a fluorescence detector with excitation at 263 nm and emission at 475 nm.

4-(2-Hydroxyethyl) piperazine-1-ethanesulphonic acid (HEPES buffer) was purchased from Gibco BRL (Paisley, UK). RPMI 1640 was purchased from Life Technologies (Paisley, UK). All other chemicals and solvents were of analytical grade and obtained either from Sigma (St. Louis, MA, USA) or from Merck (Darmstadt, Germany).

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Complete Culture Medium. Complete culture medium (CCM) was prepared by mixing 85 ml of preliminary culture medium (PCM) and 15 ml of AB-positive human serum (heat inactivated at 57°C for 30 min) and gentamicin was added to give a final concentration of 10 µg ml^{-1}.

PCM consisted of 10.43 g RPMI 1640, 5 g HEPES buffer, 2 g sodium bicarbonate, 50 mg hypoxanthine and 2 g glucose in 1000 ml of deionized water. The medium was passed through Millipores filter (0.22 µm) and kept at 4°C before use.

Drug solutions. Stock solutions of rac-MFQ, (-)-MFQ and (+)-MFQ hydrochloride were prepared by dissolving, by sonication, 20.7 mg of each of the samples in 5 ml 70° ethanol to give a final concentration of 10 mM. The solutions were passed through a 0.22 µm filter and kept at 4°C before use.

Parasite and culture conditions. The PF.IBS2 strain of *Plasmodium falciparum* resistant to chloroquine was obtained from Iranian patients in Iranshahr Research Station on Malaria, Sistan-Baluchestan province, Iran. The organisms were transferred and cultured in vitro at Department of Parasitology, School of Public Health & Institute of Public Health Research, Tehran University of Medical Sciences.

The infected blood suspensions were dispensed aseptically into 30-mm petridishes and CCM was added. The cultures were placed in an airtight candle jar and incubated at 37°C according to Trager & Gensen [11]. The culture media were changed every 24 h. Thin films were prepared and stained using 5% Giemsa in phosphate buffer (pH=7.2) for 30 min and examined to determine parasite density. When the parasitemias of the samples reached to the range of 0.5-1.5%, they were used to test drug sensitivity after synchronization.

Synchronization of the parasites. Synchronization was performed according to Lamros & Vanderberg [12]. Briefly the cell suspension of the parasites were transferred to a test tube and centrifuged at 700 g for 2 min. The supernatant was discarded and about 5 volumes of the compact cells, the 5% D-sorbitol in distilled water was added, and mixed to give a homogeneous suspension. After 12 min at 37°C the supernatant was removed and the red blood cells were cultured as usual for 8 hrs. Then the cultured parasites were transferred to 96-well plates for drug sensitivity test.

Drug sensitivity test. Tenfold serial dilutions of rac-MFQ and its enantiomers were prepared in duplicate across a 96-well plate by putting 10 µl aliquots of each drug solution into microtiter wells and adding 90 µl of CCM to give a concentration range of 10^{-9} to 10^{-4} M. 10 µl of packed infected red blood cells were added to each well. The plate was gently agitated, placed in an airtight candle jar and incubated for 48 h at 37-37.5°C with one regassing at 24 h according to Trager & Gensen [11]. After incubation time, the contents of the supernatant solution of the test wells were removed and Giemsa-stained thin film smears were made from the compact cells of each well and were examined under light microscope for the presence of asexual parasites. The experiment was performed on three independent occasions.

Data analysis. The number of parasites was counted against 1000 red blood cells. The results of the average counting for each concentration of the drugs were expressed as parasitemia. The percentage of growth inhibition was expressed according to following equation:

$$\text{growth inhibition \%} = 100 - [(\text{test}_{\text{parasitemia}} / \text{control}_{\text{parasitemia}}) \times 100]$$

The averages of percentage of growth inhibition, resulted from three independent tests, were calculated.

The 50% inhibitory concentrations were achieved by probit analysis of the log dose/percentage of growth inhibition.

RESULTS

Enantiomeric purity of the resolved enantiomers. The enantiomeric purity of the MFQ enantiomers was assayed using a modified indirect HPLC method as described above. The purity of the (-)-MFQ and (+)-MFQ was 100% and 98.6% respectively.

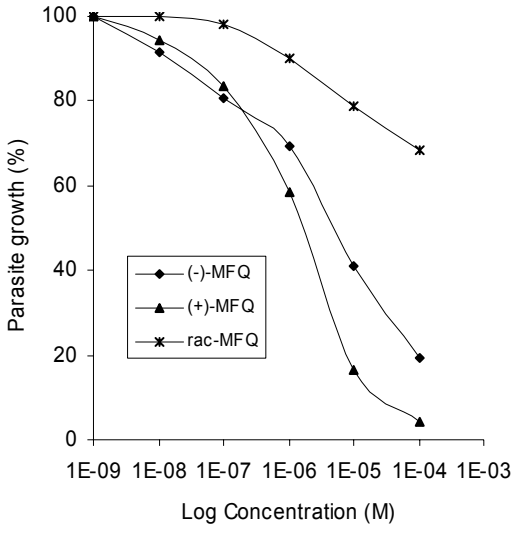

Fig 1. In vitro activity of rac-MFQ and MFQ enantiomers against *Plasmodium falciparum* PF.IBS2.

Antimalarial activity. The antimalarial activity, calculated as the percentage of growth inhibition of rac-

Table 1. The antimalarial activity of rac-MFQ and its enantiomers against Plasmodium falciparum strain PF.IBS2

	% of growth inhibition					
Form of mefloquine	**10^{-9} M**	**10^{-8} M**	**10^{-7} M**	**10^{-6} M**	**10^{-5} M**	**10^{-4} M**
rac-MFQ-HCl	0	0	1.88 ± 0.13	9.94 ± 0.49	21.1 ± 2.82	31.8 ± 3.59
(-)-(SR)-MFQ-HCl	0	8.69 ± 1.87	19.56 ± 3.11	30.78 ± 1.64	58.86 ± 4.31	80.43 ± 5.33
(+)-(RS)-MFQ-HCl	0	5.76 ± 1.78	16.66 ± 3.19	41.66 ± 5.93	83.33 ± 3.08	95.83 ± 2.29

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MFQ, (-)-MFQ and (+)-MFQ on *Plasmodium falciparum* strain PF.IBS2, is presented in Table 1 and Figure 1. No inhibition was seen for rac-MFQ and its pure enantiomers at concentrations below 10^{-7} M and 10^{-8} M respectively. Concentrations over 10^{-8} M of MFQ enantiomers inhibited the growth of *Plasmodium falciparum*. The inhibition was increased by increasing the concentration of drugs. The IC_{50} values calculated for (-)-MFQ and (+)-MFQ were 4.09±0.11 and 1.17±0.34 µM respectively which show the higher activity of (+)-MFQ compared to (-)-MFQ.

DISCUSSION

Studies carried out on the antimalarial activity of MFQ enantiomers presented various results. In an in vitro study on *Plasmodium falciparum* no stereoselective activity is shown [8]. In another investigation the (+)-isomer was more active (1.69 to 1.95 times) than (-)-isomer [9]. In the present study both enantiomers were shown to be more potent than the racemic MFQ. Furthermore, the (+)-MFQ showed higher activity in comparison to (-)-MFQ. The results of this study are in support of what reported by Karle et al [9] who found that the (+)-MFQ was 1.69 to1.95 times more active than the (-)-MFQ against *Plasmodium falciparum* strains Indochina W-2 and Sierra Leone D-6 in vitro. However the findings of this study are not consistent with the results reported by Basco et al [8] which showed equal activity for both enantiomers against Ivory Coast L-3 and Cameroon FCM 29 *Plasmodium falciparum* strains. The difference between the results of this study with other references may be due to the biochemical differences of the isolated strains or the applied techniques.

CONCLUSION

The results of this study showed that (+)-MFQ has higher activity than (-)-MFQ against *Plasmodium falciparum* strain PF.IBS2 in vitro. In one report the same results was obtained but it was not supported by others which may be due to the biochemical differences of the parasite strains or the in vitro methods used.

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