



## An *Ex-Vivo* Study on the Stereoselective Accumulation of Mefloquine Enantiomers in Human Blood Fractions

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### Abstract

Mefloquine (MFQ), as a racemic mixture is used for the prophylaxis and treatment of malaria. Stereoselective pharmacodynamic and pharmacokinetic differences have been observed for MFQ. In the present study, the human blood was spiked with racemic MFQ. The concentration of MFQ enantiomers in various blood fractions including packed erythrocyte layer, platelet rich plasma and platelet poor plasma was determined. The results showed that the ratio of (+)-MFQ was about 1.5 time higher than (-)-MFQ in packed erythrocyte layer. Results obtained from the separated erythrocytes spiked with racemic MFQ showed no significant difference between the enantiomer concentrations. It can be concluded that the stereoselective accumulation of MFQ enantiomers in erythrocytes might be in relation to protein binding or the presence of other blood cells.

*Keywords:* Mefloquine; Enantiomer; Stereoselective; Blood fractions.

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### 1. Introduction

Mefloquine (MFQ) is a chiral drug used orally as a racemate mixture for the prophylaxis and treatment of malaria caused by multiple drug resistant strains of *Plasmodium falciparum* [1]. Various studies have demonstrated highly stereoselective pharmacokinetics of this chiral drug. The peak concentration and the area under the curve (AUC) of (-)-MFQ have been significantly higher than those of (+)-MFQ in the whole blood and plasma after oral administration of

racemic MFQ [2-4]. Reverse stereoselectivity was also reported in rat. Higher concentrations of (+)-MFQ were observed in the rat plasma [5, 6], while higher concentrations of (-)-MFQ were reported in the rat brain [6]. Higher activities of (+)-MFQ against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* has been observed than (-)-MFQ, *in vitro* [7, 8].

The accumulation of racemic MFQ in erythrocytes, which is of particular interest in the prophylactic effect of this drug, has been studied previously [9, 10]. A preliminary study in rat demonstrated stereoselective accumulation of MFQ enantiomers in different

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blood fractions with a tendency of (+)-MFQ for leukocytes and (-)-MFQ for erythrocytes [5]. A stereoselective P-glycoprotein inhibition in rat brain capillary endothelial GPNT cells was also observed, and (+)-MFQ showed higher interaction than its antipode. However, in Caco-2 human cell lines, no stereoselective P-glycoprotein inhibition was observed [11]. Our literature survey showed no information regarding the stereoselective accumulation of MFQ in human blood fractions. In the present study, the stereoselective accumulation of MFQ enantiomers in various blood fractions including packed erythrocyte layer (PEL), platelet rich plasma and platelet poor plasma, was measured after the incubation of the whole blood spiked with racemic MFQ, using a previously reported stereoselective assay [12].

## 2. Materials and methods

### 2.1. Chemicals

Racemic mefloquine hydrochloride was kindly donated by Roche (Basel, Switzerland). The (+)-1-(fluorenyl) ethylchloroformate reagent (FLEC, 18 mM in acetone) and 4-(2-hydroxyethyl) piperazine-1-ethanesulphonic acid (HEPES) were purchased from Fluka (Buchs, Switzerland). The enantiomeric purity of (+)-FLEC was greater than 99.5%. The principal HEPES buffer consisted of 10 mM HEPES and 147 mM NaCl, adjusted to pH 7.4 with 0.1 M NaOH. All other solvents and chemicals were of either chromatographic or analytical reagent grade from Merck (Darmstadt, Germany).

### 2.2. Sample collection

Aliquots of 30 ml of freshly drawn blood from forearm veins of 6 healthy volunteers (3 males and 3 females) between 20 to 40 years of age were put into six heparinized (100 units/ml) test tubes. All volunteers were examined to be healthy according to their medical history and physical examinations. None of the subjects were taking any

medications for at least 2 weeks before blood collection. Each blood sample was divided into four siliconized test tubes.

### 2.3. Incubation of whole blood

Aliquots of MFQ solution (4 mg/ml in methanol) were added to each of the four blood samples to obtain a concentration range of 500, 1000, 1500 and 2000 ng/ml of racemic MFQ, which was consistent with the blood concentration ranges achieved after the administration of different oral therapeutic doses of MFQ (up to 1500 mg orally). The tubes were mixed and incubated for 20 min at room temperature for equilibration. Then an aliquot of 0.5 ml of each tube was taken for determination of MFQ enantiomers in the whole blood.

### 2.4. Separation of blood fractions

The blood fractions were prepared according to the method described by Brocks et al. [13]. The blood samples, incubated with MFQ, were centrifuged at 450 rpm for 10 min and the platelet rich plasma was carefully removed avoiding contamination with erythrocytes. An aliquot of 0.5 ml from each test tube was taken for analysis. The buffy coat layer was removed by aspiration and the erythrocyte rich suspensions were washed three times with HEPES buffer (pH 7.4) followed by successive centrifugation. The supernatant was carefully removed and the residue was resuspended in the same buffer to achieve a desired haematocrit (35%), which was accurately measured using a haematocrit centrifuge (Yankee®, Clay-Adam's Inc., New York). To collect the platelet poor plasma, the platelet rich plasma was centrifuged at 5000 rpm for 10 min, and the supernatant was removed carefully. All samples were stored at -20 °C until the stereoselective MFQ determination.

### 2.5. Incubation of separated erythrocytes

To 5 ml of freshly drawn blood from

forearm veins of volunteers (n=6) in a siliconized test tube, heparin (100 units/ml) was added and mixed. The blood samples were centrifuged for 20 min at 450 rpm and the platelet rich plasma and the buffy coat layer were removed completely. The erythrocyte layer was washed three times with HEPES buffer (pH 7.4). The residue was resuspended in the same buffer to reach a 35% haematocrit. Each sample was divided into four siliconized test tubes. The MFQ solution (4 mg/ml) was added to each test tube to obtain final concentrations of 500, 1000, 1500 and 2000 ng/ml. After incubation for 20 min at room temperature, the erythrocytes fraction was washed again with HEPES buffer three times. Then HEPES buffer was added to reach the desired haematocrit (35%) and the concentration of the MFQ enantiomers were determined.

#### 2.6. Chromatographic conditions

The chromatographic system consisted of a 510 HPLC pump, a WISP 717 autoinjector, a 486 fluorescence detector and a 476 integrator (Waters, Milford, MA, USA). The reversed-phase system consisted of a Novapak C18 (150 mm x 3.9 mm, 5  $\mu$ m) cartridge column (Waters, Milford, MA, USA) and a mobile phase of acetonitrile-water-glacial acetic acid (730:270:0.7, v/v/v) at a flow-rate of 1 ml/min. Fluorescence detection was performed using a detector with excitation at 265 nm and emission at 475 nm.

#### 2.7. Determination of mefloquine enantiomers

The concentrations of MFQ enantiomers in different blood fractions were measured using a stereoselective HPLC method [12] with slight modifications. Briefly, frozen blood samples were thawed and were allowed to adjust to the room temperature. A 500  $\mu$ l aliquot of each sample was placed into a siliconized test tube and 500  $\mu$ l of sodium hydroxide 0.2 N and 5 ml of methyl tert-butylether were added successively. The test

tube was vortex-mixed and the organic layer was transferred to a clean test tube and evaporated to dryness at 50 °C under nitrogen gas. One hundred  $\mu$ l of (+)-FLEC reagent (36 mM in acetonitrile) and 50  $\mu$ l of borate buffer 43  $\mu$ M (prepared by dissolving 0.26 g boric acid and 0.32 g potassium chloride in 100 ml distilled water, adjusted to pH 8.5 with 1 M sodium hydroxide) were added to the residue. The mixture was vortexed and was allowed to react at the room temperature for 40 min. After centrifugation at 2000 rpm for 10 min, 20  $\mu$ l of the solution were loaded into the HPLC column.

#### 2.8. Statistical analysis

The results reported as mean $\pm$ SD and the difference between the concentrations of MFQ enantiomers in different blood fractions were assessed using student's two sided t-test at  $\alpha=0.05$  level of significance.

### 3. Results

Concentrations of MFQ enantiomers in different blood fractions and their (+)/(-) ratios are presented in Table 1. Accumulation of MFQ enantiomers in the platelet poor plasma and the platelet rich plasma was enhanced by increasing the concentration of spiked rac-MFQ. The concentration of MFQ enantiomers in the packed erythrocyte layer approximately showed such an enhancing manner. No significant difference was seen between MFQ enantiomers in the platelet poor plasma and the platelet rich plasma fractions. A significantly higher concentration of (+)-MFQ ( $p<0.004$ ) was observed in PEL in comparison to (-)-MFQ. Since the erythrocytes are considered as the site of antimalarial effect of MFQ, a new sample of separated PEL was spiked with rac-MFQ and was processed as described above. The concentration of MFQ enantiomers was determined and the (+)/(-) ratios of MFQ in PEL samples spiked with rac-MFQ after separation from intact blood, are presented in

**Table1:** Concentrations of mefloquine enantiomers (ng/ml) in 0.5 ml of various blood fractions after incubation of blood samples (n=6) spiked with racemic mefloquine (500-2000 ng/ml).

No.	(+)-Mefloquine			(-)-Mefloquine			Total Mefloquine			Ratio (+)/(-)
	Plasma	Whole Blood	Red Blood Cells	Plasma	Whole Blood	Red Blood Cells	Plasma	Whole Blood	Red Blood Cells	
1	1500	1800	1200	1200	1500	1800	2700	3300	3600	1.5
2	1800	2100	1500	1500	1800	2100	3300	3900	4200	1.6
3	2100	2400	1800	1800	2100	2400	3900	4500	4800	1.7
4	2400	2700	2100	2100	2400	2700	4500	5100	5400	1.8

\*Significantly different from the other enantiomer..

Table 1. The (+)/(-) ratios of the MFQ enantiomers in the PEL spiked before separation is in the range of 1.5-1.8, while this range is 1.1-1.4 in PEL spiked after separation. No significant difference was seen between the MFQ enantiomers when PEL was spiked with rac-MFQ after separation from other fractions. Therefore, the higher accumulation of (+)-MFQ in PEL when the blood sample is spiked with MFQ before separation of blood fractions (Table 1) may be due to other fractions such as platelets, leukocytes and/or proteins.

#### 4. Discussion

As discussed before no *in vitro* or *in vivo* study has so far been carried out on the accumulation of the MFQ enantiomers in different blood fractions. Stereoselective accumulation of the MFQ enantiomers in blood fractions of rat after oral administration of rac-MFQ is reported by the same authors with higher concentrations of (-)-MFQ in PEL [5]. So, an interspecies difference is observed between MFQ enantiomer concentrations in blood fractions.

Pharmacokinetic studies of the MFQ enantiomers have shown significantly higher concentrations of (-)-MFQ in the human plasma and the whole blood [2-4]. Reverse stereoselectivity is reported in the rat plasma

[5, 6]. The results of this study showed no specific tendency of the MFQ enantiomers for different blood fractions *in vitro*. It can be concluded that no reasonable interaction can be defined for the MFQ enantiomers with blood fractions at *in vivo* studies. Different concentrations of the MFQ enantiomers after oral administration of racemic MFQ and interspecies differences might be explained by one or more stereoselective pharmacokinetics processes such as absorption, excretion, metabolism and distribution. Further studies on the pharmacokinetics of the MFQ enantiomers are needed to elucidate the overall pathway of this drug in the human body.

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