# A PRELIMINARY STUDY OF STEREOSELECTIVITY OF MEFLOQUINE ENANTIOMERS IN RAT

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*Abstract – Stereoselectivity of mefloquine enantiomers were studied in rats after oral administration of a single 50mg/Kg dose of the racemate. Pharmacokinetic parameters of (+)-(RS)-MFQ in blood and plasma showed no significant difference. The concentration, AUC (0-∞), CL/F and Vd/F of (+)-(RS)-enantiomer in blood were significantly higher than those for the (-)-(SR)-enantiomer. The results obtained from this study showed a reverse stereoselectivity of MFQ as compared with what reported for human. A very low amount of enantiomers excreted in urine and the excretion was not stereoselective. A preliminary study in different blood fractions showed that the accumulation of MFQ enantiomers in blood cells is stereoselective with a tendency of (+)-(RS)-enatiomer for leukocytes and (-)-(SR)-enantiomer for erythrocytes.*
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*Key words: Mefloquine, enantiomer, stereoselectivity, rat*

## INTRODUCTION

Mefloquine(MFQ),rac-erythro- α -(2-piperidyl)-2, 8-bis (trifluoromethyl)-4-quinolinemethanol, is a chiral drug which is administered orally as a racemate mixture for prophylaxis and treatment of malaria caused by multiple drug-resistant strains of Plasmodium falciparum. There are conflicting reports about the antimalarial activity of MFQ enantiomers. In some reports no significant difference is observed between antimalarial activities of enantiomers (1, 2). In one report (3) the (+)-(RS) -enantiomer of MFQ was 1.69 to 1.81 times more active than the (-)-(SR)-enantiomer aganist chloroquine- sensitive and chloroquine-resistant strains of Plasmodium falciparum in vitro. Pharmacokinetic studies of MFQ enantiomers in human have shown high stereoselectivity. The peak concentration and the area under the curve (AUC) of the (-)-(SR)-enantiomer have been significantly higher than those of (+)-(RS)-MFQ (4-6).

Literature survey showed that no reports have so far been given on the enantioselective protein binding and accumulation of MFQ enantiomers in erythrocytes. Recently, our laboratory reported a sensitive indirect high performance liquid chromatography (HPLC) method for determination of MFQ enantiomers in biological fluids (7). In the present report, this method was used to study the stereoselective parameters of MFQ enantiomers in rats.

## MATERIALS AND METHODS

### *Chemicals and Reagents*

Racemic MFQ-HCl was purchased from Roche (Basel, Switzerland). Internal standard, rac-bupranolol (IS) was from Logeais, Issy-les-Molineaux, France. The derivatizing reagent, (+)-(S)-naphthylethylisocyanate (NEIC) was purchased from Aldrich (Milwaukee, WI, U.S.A.). The optical purity of the reagent was higher than 99.5%. Hexane, chloroform, methanol and isopropanol were HPLC grade products from Fisher Scientific (Fair Lawn, NJ, U.S.A.). All other chemicals and solvents were of analytical reagent grade and used without any further purification.

### *Sample Collection*

In order to find the interval times of blood sampling, two male Sprague-Dawley rats underwent surgical cannulation of the right jugular vein. The animals were initially anesthetized with diethylether and then maintained on methoxyflurane (Metofane, Pitman- Moore Ltd, Missisauga, Ontario, Canada). The animals were allowed to recover overnight. In the morning, the rats were housed individually in metabolic cages. According to the effective dose of 25mg/Kg in human a single dose of 50mg/Kg racemic MFQ was administered orally to them after 12 hours of fasting

and successive blood samples were collected in heparinized test tubes from the catheter. The catheter was flushed with heparin in saline (100 units/ml) after each sample. Plasma was separated by centrifugation at 1800g for 3 min using a Fisher Model 235A microcentrifuge (Fisher Scientific, Edmonton, Canada). The concentration of MFQ enantiomers in blood and plasma samples and the appropriate interval times of blood sampling were determined. Four rats were used with the same procedure and blood samples (600$\mu$l) were collected after 1, 6, 24, 36, 48 and 72 hours. Blood and plasma samples were stored at -20°C before analysis. The urine output was collected by an interval time of 12h till 72 hours and kept at -20°C before analysis. The concentration of MFQ enantiomers in different blood fractions was determined. The concentration of the (+)-(RS)-enantiomer was markedly higher than (-)-(SR)-enantiomer in blood (P=0.026), BCL (P=0.004) and REP (P=0.01) (Table 3). There was also a trend for a higher concentration of (+)-(RS)-enantiomer over (-)-(SR)-enantiomer in PRP (P=0.068). No significant difference was observed between enantiomer concentrations in PPP (P=0.118). The concentration of the (+)-(RS)-enantiomer in all blood fractions were higher than PPP (Table 3). The concentration of (+) - (RS) - enantiomer in BCL was significantly higher than PPP and PRP (P=0.014, P=0.023). The concentration of (-)-(SR)-enantiomer in BCL and REP was significantly higher (P=0.027, P=0.041) than PPP fraction. The concentration of (-)-(SR)- enantiomer in REP was higher (P=0.064) than whole blood. A trend for higher concentration of (-) - (SR) - enantiomer in REP over BCL (P = 0.084) was observed.

For the in vivo determination of disposition of MFQ enantiomers in blood cells, rac-MFQ was administered to rats (n=3) orally (50 mg/Kg). After 12 hours the animals were anesthetized by the above mentioned method and their whole blood (5-7 ml) was collected via cardiac puncture into EDTA vacutainer tubes (Becton Dickinson, Rutherford, NJ). The blood fractions were prepared according to D.R. Brocks and coworkers (8). After centrifugation the blood sample at 150g for 10 minutes, the top layer, containing the platelet-rich plasma (PRP), was collected. The buffy coat layer (BCL) and the bottom layer which is rich in erythrocytes and platelets (REP) were separated. To collect platelet-poor plasma (PPP), the platelet-rich plasma was centrifuged again at 1800g for 10 minutes. All samples were stored at -20°C befor analysis.

The MFQ enantiomers were determined using our perviously reported HPLC technique (7). Briefly this method involves the extraction of rac-MFQ and internal standard (bupranolol) from basified blood or plasma with hexane-isopropanol (95:5 v/v), evaporation the organic layer and then derivatization with(+) -(S) - naphthy- lethylisocyante. The diastereomers was determined using a Partisil 5 (Silica) column (250×4.6 mm, Phenomenex, Torrance, CA, U.S.A.) and a mobile phase of hexane- chlorofom- methanol (74: 25: 1, v/v/v) pumping at a flow-rate of 1ml/min. Using a UV detector at 282 nm the detection limit of the analysis method was 0.04 $\mu$g/ml for each enantiomer in 200 $\mu$l of blood or plasma. The whole blood and plasma concentrations of the enantiomers were plotted versus time. The elimination rate ($\beta$) was calculated from the terminal portion of the log concentration versus time curves. The area under the concentration versus time curve, AUC (0-72), was calculated using the linear trapezoidal rule. The AUC (0-∞) was calculated as AUC (0-72)+C72/$\beta$, where C72 was the last concentration measured. Half-life ($t_{1/2}$) was calculated from the ratio 0.693/$\beta$. The oral clearance was determined from the equation CL/F=Dose/AUC (0-∞), and the apparent volume of distribution (Vd/F) from Dose/$\beta$×AUC (0-∞). The difference between pharmacokinetic parameters and concentration of MFQ enantiomers in different farctions of blood were assessed using Student's two sided t-test at $\alpha$=0.05 level of significance.

## RESULTS

The average plasma and whole blood concentration time profiles of (+)-(RS)-MFQ and (-)-(SR)-MFQ after oral administration of 50 mg/Kg of rac-MFQ to rats are presented in Fig. 1. Blood concentration of MFQ enantiomers exhibited significant

stereoselectivity. The blood concentrations of (+)-(RS)-MFQ, were significantly (P<0.03) greater than (-)-(SR)-MFQ at most measured times except for 1h (Table 1 and Fig. 1.). Pharmacokinetic parameters of MFQ enantiomers are shown in Table 2. Both enantiomers showed thier maximum concentration at 6h. Significant difference in AUC (0-∞) (+/-ratio=2.3), CL/F and Vd/F was observed for MFQ enantiomers. Concentration of (+) - (RS) - MFQ in plasma was significantly (P<0.003) higher than (-) - (SR) - MFQ at 6h (Fig. 1). At other time points, this significancy could not be calculated due to large variability in plasma concentration of the (+)-(RS)-MFQ and low concentrations of (-)-(SR)- MFQ which were undetectable or close to the detection limit of the method. Therefore pharmacokinetic parameters of

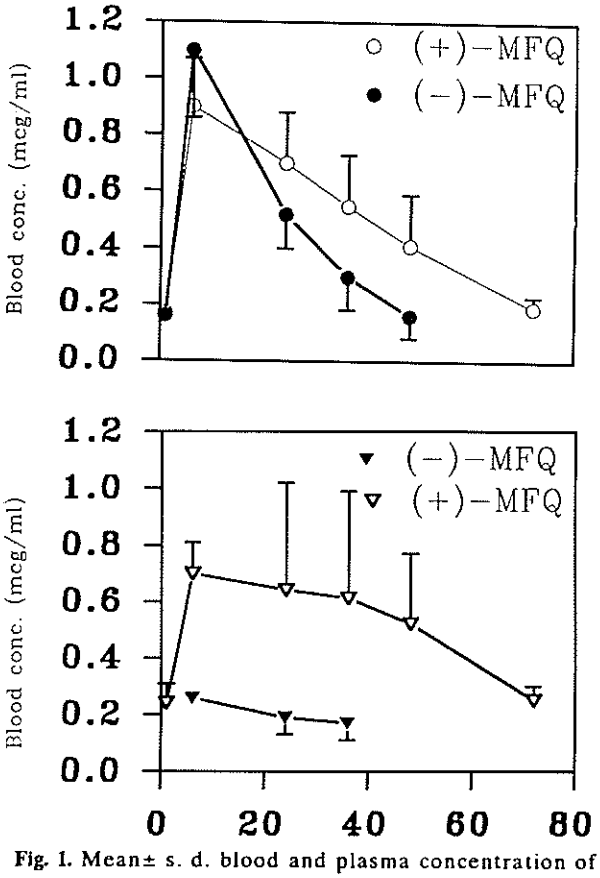


Fig. 1. Mean± s. d. blood and plasma concentration of mefloquine (MFQ) enantiomers in rat (n=4), following a single oral dose of 50mg/Kg rac-MFQ.

Table 1. Mean ± s. d. concentration (μg/ml) of mefloquine (MFQ) enantiomers in rat blood and plasma (n=4).

| | Blood | | Plasma | |
|---|---|---|---|---|
| Time(h) | (+)-Mefloquine | (-)-Mefloquine | (+)-Mefloquine | (-)-Mefloquine |
| 1 | 0.18±0.01 | 0.17±0.01 | 0.45±0.30 | 0.27±0.03 |
| 6 | 0.79±0.23* | 0.55±0.18 | 1.01±0.51* | 0.40±0.23 |
| 24 | 0.65±0.16* | 0.33±0.07 | 0.86±0.47 | 0.27±0.14 |
| 36 | 0.51±0.16* | 0.22±0.07 | 0.79±0.42 | 0.19±0.05 |
| 48 | 0.40±0.14 | 0.18±0.05 | 0.64±0.29 | |
| 72 | 0.22±0.05 | | 0.35±0.17 | |

* significantly different (0.003 < P<0.04, from corresponding (-) - enantiomer

Table 2. Pharmacokinetic parameters of mefloquine enantiomers in blood and plasma following 50 mg/kg single oral dose of rac-mefloquine to rats (n=4).

| Mefloquine enantiomer | Cmax (μg/ml) | Tmax (h) | AUC(0-∞) (mg.h/L) | $t_{1/2}$ (h) | CL/F (L/h/kg) | Vd/f (L/kg) |
|---|---|---|---|---|---|---|
| **Blood** | | | | | | |
| (+)- Mefloquine | 0.79±0.23 | 6±0.00 | 44.94±10.60* | 25.93±7.29 | 0.19±0.05* | 7.09±2.40* |
| (-)-Mefloquine | 0.55±0.18 | 6±0.00 | 22.48±5.78 | 21.62±7.72 | 0.38±0.10 | 11.28±2.25 |
| **Plasma** | | | | | | |
| (+)- Mefloquine | 1.01±0.51 | 6±0.00 | 57.57±20.38 | 29.23±10.91 | 0.15±0.06 | 6.82±4.50 |

* Significantly different (0.01<P<0.04) from corresponding (-)-enantiomer

Table 3. Mean ± s.d. concentration (μg/ml) of mefloquine (MFQ) enantiomers in rat (n=3) blood fractions 12 h after a single oral dose of 50 mg/kg rac-MFQ.

| Biological material | (+)-Mefloquine (mean±s.d.) | (-)-Mefloquine (mean±s.d.) | (+)/(-)ratio (mean±s.d.) |
|---|---|---|---|
| Platelet-poor plasma | 0.49±0.04 | 0.32±0.08 | 1.58±0.28 |
| Platelet-rich plasma | 0.62±0.09 | 0.43±0.14 | 1.48±0.19 |
| Buffy coat layer | 0.89±0.04 | 0.55±0.06 | 1.63±0.08 |
| Spun erythrocytes and platelets | 0.88±0.16 | 0.70±0.14 | 1.27±0.03 |
| Blood | 0.72±0.12 | 0.49±0.09 | 1.47±0.08 |

(-)-(SR)-MFQ based on plasma concentration could not be calculated. No significant difference was observed between concentration, AUC (0-∞), CL/F and Vd/F for (+)-(RS)-MFQ in blood and plasma. On the other hand, the blood concentrations of (-) - (SR) - MFQ were significantly higher than its plasma concentrations at 6h ($P<0.007$) and 24h ($P<0.014$).

## DISCUSSION

Previous reports in human subjects have shown that after oral administration of racemic MFQ, the whole blood and plasma concentrations of the (-)-(SR)-MFQ were higher than (+)-(RS)-MFQ (4-6). However, the findings of this study showed a reverse stereoselectivity of MFQ enantiomers in rats as compared to human data. These results have shown that stereoselective pharmacokinetics of MFQ enantiomers might be species-dependent. The total output of MFQ enantiomers in urine after 72 hours was about 0.008mg for (+)-(RS)-MFQ and 0.007mg for (-)-(SR)-MFQ. Therefore the recovery of the rac-MFQ initial dose excreted in 72 hours as unchanged drug was quite insignificant (0.06%). Stereoselectivity in renal excretion was not observed.

Erythocytes were known to concentrate rac-MFQ (9, 10), but, according to our knowledge, the stereoselectivity of the accumulation of MFQ has not been studied yet. In this study the disposition and stereoselectivity of the MFQ enantiomers in different rat blood farctions 12 hours after oral administration of 50 mg/Kg of racemic mefloquine was studied. Comparing the concentration of MFQ enantiomers in blood fractions results showed the tendency of accumulation of both enantiomers in all blood cells with a higher tendency of accumulation of (+) - (RS) - enantiomer in leukocytes and (-)- (SR)-enantiomer in erythocytes. The stereoselective accumulation of MFQ enantiomers in blood cells might be in relation to stereoselective protein binding of enantiomers, as we have observed a concentration dependent ratio of (+) - (RS) - enantiomer in blood over plasma. A trend for a higher blood/plasma concentration ratio around maximum concentration and lower ratio in lower concentrations of (+)-(RS)-enantiomer was observed which may be due to a stereoseletive saturation of the disposition pathway. The stereoselective metabolism can not also be excluded which remains to be studied. In conclusion the concentrations of (+)-(RS)-MFQ in blood and plasma were higher than (-)-(SR)-MFQ in rats. These results showed a reverse stereoselectivity in rats as compared with those reported for human. Stereoselective accumulation in rat blood cells was also observed.

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