Enantioselective pharmacokinetics of mefloquine during long-term intake of the prophylactic dose

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Aims To investigate the kinetics of the (+)RS- and (–)SR-enantiomers and the carboxylic acid metabolite (MMQ) of the antimalarial drug mefloquine (MQ) in healthy volunteers.

Methods Ten subjects of which three were poor metabolizers of debrisoquine received racemic MQ 250 mg once weekly for 16 weeks. The kinetics were followed in plasma and urine and evaluated by individual kinetic modelling as well as by a nonparametric population kinetic method.

Results A two-compartment model adequately described the disposition of (+)RS-MQ. CL/F was $6.5 \pm 1.8 \text{ l h}^{-1}$, V_{ss}/F $815 \pm 165 \text{ l}$, and k $0.0081 \pm 0.0023 \text{ h}^{-1}$. The kinetics of (–)SR-MQ were time- and/or concentration dependent with a lower oral clearance (0.92 ± 0.25 vs $2.14 \pm 0.63 \text{ l h}^{-1}$, 95% CI for the difference $0.86\text{--}1.60 \text{ l h}^{-1}$) and a longer half-life (345 vs 185 h , 95% CI for the difference $47\text{--}291 \text{ h}$) after the last dose compared with the first dose. Clearance of (+)RS-MQ and (–)SR-MQ was significantly correlated within subjects ($r=0.69$, $P<0.05$). Urinary recovery of unchanged substance was 8.7% for (+)RS-MQ and 12.3% for (–)SR-MQ. The elimination of MMQ was disposition rate-limited and not determined by its rate of formation. Poor metabolizers of debrisoquine did not differ from extensive metabolizers in the kinetics of any compound.

Conclusions The MQ enantiomers differ extensively in their disposition both with regard to parameter values and the kinetic stability over time during repeated dosing with racemic MQ. Kinetic modelling of racemic MQ is therefore inadequate.

Keywords: mefloquine, pharmacokinetics, enantiomers, population pharmacokinetics

Introduction

Mefloquine (MQ), a quinoline methanol chemically related to quinine, is an important drug for malaria prophylaxis and treatment of multi-drug resistant *P. falciparum* malaria. The available preparation is a racemate of the (+)RS-MQ and (–)SR-MQ enantiomers. Both enantiomers are active against *P. falciparum* *in vitro* [1] although a slightly higher activity for (+)RS-MQ has been reported [2]. A major problem with MQ is the occurrence of neuropsychiatric adverse reactions, in particular after therapeutic doses [3]. It has not been clarified whether the potential to cause adverse reactions differs between the enantiomers. MQ is a local irritant that cannot be given intravenously. Hence, absolute bioavailability and systemic clearance are not known. The metabolism of MQ is not well characterised. At steady-state, an average of 9% of the dose is excreted unchanged in urine and 4% as the carboxylic acid metabolite (MMQ) [4]. MMQ is regarded as the main metabolite in man but it has no significant antimalarial effect *in vitro* [5].

A recent pharmacokinetic study during prophylactic intake of MQ (250 mg once weekly) demonstrated a significantly longer mean half-life of (–)SR-MQ compared

with (+)RS-MQ (430 vs 172 h) in plasma [6]. The mean difference in AUC at steady-state was 6.6-fold. Similar findings have been reported in Thai volunteers given a single dose of 750 mg MQ [7].

The aims of the present study were to investigate the enantioselective kinetics of MQ during long-term prophylaxis including the early phase after drug intake, the renal excretion of the enantiomers and to define any difference between poor and extensive metabolizers of debrisoquine in the elimination of the MQ enantiomers. For determination of the typical kinetic characteristics for (+)RS-MQ and (–)SR-MQ in this population, we performed a population analysis using the nonparametric NPEM method [8]. Compared with the standard two-stage method, more accurate estimates of the distribution of kinetic parameters in a population can be obtained with the population methodology as it takes into account the quality of the data from each subject and the covariance between the parameters.

Methods

Subjects

Ten healthy adult Caucasian volunteers (mean age 42 years, range 29–50 years, six females and four males) participated in the study. Seven were extensive and three were poor

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hydroxylators of debrisoquine according to a previous phenotyping. Before inclusion, a health check-up including medical history, physical examination and blood chemistry was carried out. One subject (1) was on continuous antihistamine treatment (loratadine) and one subject (2) was on anticonceptive therapy (Depot-Provera injection every 3 months). The study was approved by the local ethics committee at Huddinge University Hospital (11/92).

Study design

Racemic mefloquine (Lariam[®], Hoffman La Roche, Switzerland) was given in the adult prophylactic dose of 250 mg once weekly for a total of 16 weeks. The aim was to reach steady-state conditions in all subjects. All volunteers took the first and last MQ dose on an empty stomach (fasting for at least 10 h) and remained fasting during the first 3 h after intake. Food was allowed with the other doses. The subjects filled out a standardized questionnaire about typical adverse effects of mefloquine once weekly during the study. They also participated in a separate study of hearing, vestibular functions and postural control. Details of the tolerability are presented in that paper [9].

Venous blood for drug analyses was drawn in connection with the first and last doses at 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 168 h post dose. After the last dose, additional samples were drawn at 14, 21, 28, 35 and 42 days. A single sample was taken once weekly immediately before intake of doses 3–16. The Vacutainer system with tubes containing ethylenediamine tetraacetate (EDTA) as anticoagulant was used. Samples were centrifuged within 30 min and plasma was frozen for later drug analysis.

Urine was collected in 24 h intervals during 7 days after intake of the last dose.

Analytical methods

Plasma and urine levels of (+)RS-MQ and (–)SR-MQ MQ and MMQ (2,8-bis(trifluoromethyl)-4-quinoline carboxylic acid) were determined by h.p.l.c. [10, 11]. The limits of determination related to the free base were 0.25 μM [95 $\mu\text{g l}^{-1}$] for MMQ, 0.01 μM [3.8 $\mu\text{g l}^{-1}$] for (+)RS-MQ and 0.05 μM [19 $\mu\text{g l}^{-1}$] for (–)SR-MQ. For the enantiomers, the intra- and inter-assay coefficients of variation were below 10% within the range of close to all concentrations measured in this study. A number of measured concentrations for (+)RS-MQ were at the above stated limit of determination at which the intra- and inter-assay coefficients of variation were between 10% and 17%.

Pharmacokinetic methods

Separate analyses were carried out for the two enantiomers and the MMQ metabolite. The software PCNONLIN was used for individual modelling of the concentration over time. The parameter values were evaluated using the kinetic model that gave the best fit to the data according to Akaike's information criterion, AIC [12]. All data for each subject were evaluated in one sequence. Data were weighted

according to the inverse of the concentration. The kinetic parameter k for MMQ was determined from data after the last drug intake.

In the population kinetic analyses, all data for each enantiomer were pooled and evaluated separately with the NPEM-method. An error model was first defined based on the standard deviation of the analytical method at different concentrations. We tested both one- and two-compartment pharmacokinetic models and compared the outcomes.

The renal excretion of unchanged (+)RS-MQ and (–)SR-MQ as a fraction of the administered dose was calculated from the total amount excreted during one dose interval at steady-state.

Statistical methods

Parametric correlation analyses were carried out for the clearance of (+)RS-MQ *vs* the clearance of (–)SR-MQ, as well as for the volume of distribution of (+)RS-MQ *vs* (–)SR-MQ. The 95% confidence interval (CI) for the difference between groups or occasions was calculated when appropriate. The distribution of kinetic parameter values among PMs and EMs of debrisoquine, respectively, was compared using the Mann-Whitney U test. A *P* value below 0.05 was considered significant.

Results

Individual modelling

For the (+)RS-MQ enantiomer, a two-compartment model was superior in 9/10 cases when compared with a one-compartment model. The mean value for AIC in all subjects was -97.2 with a one-compartment model, and -111.9 with a two-compartment model. Individual parameter values are presented in Table 1. The kinetics were stable over time with a similar quality of the fits after the first and after the last dose.

The kinetic behaviour of the (–)SR-MQ enantiomer was highly erratic with a higher mean relative peak concentration and a longer half-life after the last dose compared with the first dose (Table 1). We also found fluctuations in the concentrations of (–)SR-MQ during the first days after intake of both the first and the last doses, as shown in Figure 1. We were unable to include all of these phenomena in a general model and therefore used a conventional approach with a one-compartment model which most adequately described the data. According to AIC, a one-compartment model was superior in 7/10 cases (mean value for AIC was 30.97 for a one-compartment model and 31.72 for a two-compartment model). Due to the change in kinetics over time for (–)SR-MQ, three evaluations were conducted with different data sets: one with all data in one sequence, one with the data after the first dose only (up to the administration of the second dose), and one with the data after the last dose only. The half-life for the terminal elimination of (–)SR-MQ after the last dose was accurately estimated despite using a one-compartment model, as the concentration was determined on several occasions up to 6 weeks post dose intake. The results of all three evaluations are shown in Table 1.

Table 1 Individual and population values of the kinetic parameters for the (+)RS and (–)SR enantiomers of mefloquine and the carboxylic acid metabolite MMQ.

Subject	(+)RS-MQ						(–)SR-MQ				MMQ	
	k_a (h^{-1})	k_{12} (h^{-1})	k_{21} (h^{-1})	V_{ss}/F (l)	k (h^{-1})	CL/F ($l\ h^{-1}$)	k_a (h^{-1})	V/F (l)	k (h^{-1})	CL/F ($l\ h^{-1}$)	k (h^{-1})	Trough concentration week 15 (μM)
1	0.14	0.017	0.034	1125	0.0075	8.4	0.27	659	0.0017	1.10	0.0014	7.4
2	0.15	0.061	0.044	635	0.011	7.1	0.47	645	0.0013	0.87	0.0014	8.0
3	0.17	0.050	0.054	975	0.0051	5.0	0.69	721	0.0016	1.16	0.0013	4.4
4	0.30	0.11	0.080	540	0.0074	4.0	0.78	383	0.0016	0.61	0.0013	11.4
5*	0.23	0.016	0.022	690	0.0057	3.9	0.89	473	0.0010	0.49	0.0008	17.0
6	0.15	0.063	0.040	615	0.0095	5.8	0.54	523	0.0021	1.08	0.0015	8.9
7	0.27	0.031	0.045	790	0.0076	6.0	0.57	508	0.0019	0.97	0.0014	11.3
8	0.52	0.013	0.022	1075	0.0033	3.5	1.42	604	0.0012	0.71	0.0010	4.0
9*	0.19	0.029	0.038	830	0.0063	5.2	0.57	587	0.0018	1.07	0.0019	6.4
10*	0.14	0.041	0.051	720	0.011	7.6	0.39	560	0.0023	1.31	0.0018	19.3
Mean (s.d.)	0.22	0.043	0.043	800	0.0074	5.7	0.66	565	0.0017	0.94	0.0014	9.8
all data	(0.12)	(0.029)	(0.017)	(200)	(0.0025)	(1.7)	(0.32)	(100)	(0.0004)	(0.26)	(0.0003)	(5.0)
Mean first dose							0.52	600	0.0037	2.14		
Mean last dose							(0.24)	(160)	(0.0011)	(0.63)		
Population mean	0.15	0.034	0.032	815	0.0081	6.5	0.39	645	0.0016	1.1		
	(0.04)	(0.014)	(0.013)	(165)	(0.0023)	(1.8)	(0.15)	(145)	(0.0003)	(0.30)		

(* = PM of debrisoquine).

Figure 1 Concentration-time curve for (–)SR-MQ in subject 6 during 7 days after the last dose intake at week 16.

The analyses show that the kinetics of the two enantiomers differ considerably. The maximal observed concentration (C_{max}) after the first dose was higher for (–)SR-MQ than for (+)RS-MQ (0.65 ± 0.16 vs 0.30 ± 0.06 μM , 95% CI for the difference 0.28–0.43 μM). The mean clearance of (+)RS-MQ at steady-state was on average six times higher than that of (–)SR-MQ (5.7 ± 1.7 vs 0.94 ± 0.26 $l\ h^{-1}$, 95% CI for the difference 3.6–5.8 $l\ h^{-1}$) and the mean terminal half-life was much longer for (–)SR-MQ than for (+)RS-MQ (433 vs 94 h, 95% CI for the difference 267–408 h).

Clearance of (–)SR-MQ decreased more than twofold

over time from the first to the last dose and the mean half-life increased from 185 h to 345 h. The difference in clearance between week 1 and week 16 for (–)SR-MQ was $1.22\ l\ h^{-1}$ (95% CI 0.86–1.60 $l\ h^{-1}$), the difference in V/F was $120\ l$ (95% CI 8–232 l), and the difference in k was $0.0017\ h^{-1}$ (95% CI 0.0009–0.0026 h^{-1}).

Within subject comparisons showed that the oral clearances of (–)SR-MQ and (+)RS-MQ were significantly correlated ($r=0.69$, $P<0.05$). The same was found for V/F ($r=0.66$, $P<0.05$). CL/F and V/F were not correlated for any of the enantiomers.

The concentration of MMQ increased continuously during the first 8 weeks with minor fluctuations during the dose interval. The values of k (Table 1) were lower than the corresponding values for both (+)RS-MQ and (–)SR-MQ. Hence, elimination of MMQ is disposition rate-limited and not determined by its rate of formation. Observed trough values during week 15 were in all cases higher than the corresponding values for (+)RS-MQ and (–)SR-MQ. Poor metabolizers of debrisoquine did not differ from extensive metabolizers in any of the kinetic parameters for (+)RS-MQ, (–)SR-MQ or the MMQ metabolite, according to the Mann-Whitney U test. However, the statistical power of this comparison is low due to the limited number of subjects in the study. Oral clearance of (+)RS-MQ was $5.6 \pm 1.86\ l\ h^{-1}$ in PMs and $5.7 \pm 1.73\ l\ h^{-1}$ in EMs. The corresponding values for V_{ss} were $747 \pm 72.6\ l$ and $822 \pm 236\ l$, respectively. The values for (–)SR-MQ when fitting all data in one sequence were in PMs $CL/F\ 0.95 \pm 0.42\ l\ h^{-1}$ and $V/F\ 540 \pm 59.6\ l$, and in EMs $CL/F\ 0.93 \pm 0.21\ l\ h^{-1}$ and $V/F\ 577 \pm 114\ l$.

Population kinetic evaluation

A two-compartment model was superior to describe (+)RS-MQ kinetics as shown by the value of the log-likelihood compared with a one-compartment model (-2332 vs -2618). This was true also for (–)SR-MQ with a minor difference in the log-likelihood (-2791 vs -2872). We chose a one-compartment model for (–)SR-MQ due to the difficulties to describe adequately the concentration-time curve for (–)SR-MQ with a two-compartment model. Values of the population kinetic parameters are included in Table 1. Figure 2 shows simulated concentration curves for each enantiomer based on the population kinetic parameters found in this study, together with individual concentration curves.

Renal excretion

The amount excreted unchanged during one dose interval at steady-state (week 15) was 28.8 ± 6.6 (range 15.4–36.4) μmol of (+)RS-MQ and 40.6 ± 12.3 (range 24.2–63.2) μmol of (–)SR-MQ. The 95% CI for the difference was 4.5–19.2 μmol . Expressed as a fraction of the administered dose, the mean amount of (+)RS-MQ was 8.7% and of (–)SR-MQ 12.3%. There was a trend to correlation between the renal excretion of the enantiomers ($r=0.55$, $P<0.1$). The true renal clearance could not be calculated due to the very long half-life of both enantiomers, making a regression of plasma AUC against amount excreted in urine very uncertain.

Discussion

In a previous study where stereoselective kinetics of MQ enantiomers were shown, the sampling schedule did not allow compartmental modelling or characterisation of the early phase after drug intake [6]. New findings in the present study are the time-dependent kinetics of (–)SR-MQ, the possible enterohepatic recirculation of (–)SR-MQ, the within subject correlation of both V/F and CL/F for the enantiomers and the likely lack of influence of the CYP2D6 phenotype on the kinetics of (+)RS-MQ, (–)SR-MQ and MMQ. It is obvious that modelling of racemic MQ is inadequate as the enantiomers differ not only in kinetic parameters but also in other aspects of their disposition.

The (+)RS-MQ-enantiomer fitted well into a two-compartment model. We obtained similar results with the two-stage method based on individual results and the population approach (Table 1).

The (–)SR-MQ-enantiomer showed an unexpected kinetic behaviour. The more than double mean peak concentration after the first dose compared with (+)RS-MQ indicates that the bioavailability of (+)RS-MQ may be low. The bioavailability of MQ is unknown as an intravenous formulation of *rac* MQ is unavailable. We have no clear explanation for the higher relative peak concentration of (–)SR-MQ after the last dose compared with the first dose. A capacity limited first-pass metabolism becoming saturated at higher systemic concentrations is one possibility, which would be in agreement with the observed longer terminal elimination half-life at steady-state. Pronounced fluctuations

of the (–)SR-MQ concentration during the first days after intake was a common observation. Both enterohepatic recirculation and extended irregular absorption may contribute to this. From observations in the rat, it has been proposed that MQ undergoes enterohepatic circulation as well as metabolism in the gastrointestinal wall [13]. The high within subject correlation of both CL/F and V/F between (+)RS-MQ and (–)SR-MQ suggests that the enantiomers to some extent use similar enzymatic pathways in their metabolism. If this is true, however, the affinity of the most important isozymes must be higher since (+)RS-MQ is cleared much more efficiently.

Approximately 10% of the total administered *rac* MQ was excreted unchanged in urine with a slightly higher value for (–)SR-MQ than for (+)RS-MQ. If the bioavailability of the MQ enantiomers is less than 100%, renal clearance will account for a larger fraction of the total clearance than the approximately 10% estimated in this study. This implies that renal function may be an important factor when dosing MQ for long periods. A reduction in the clearance of the enantiomers will result in a prolonged half-life. Due to the very long half-life of MQ in subjects with normal renal function, an extended dosing period is required to detect the effects of a moderate reduction in clearance. The kinetics of MQ have been studied in two haemodialysis patients receiving the malaria prophylaxis dose [14]. One patient had similar concentrations of MQ after the dose intake at week 6 and at week 7, suggesting that steady-state was attained within 7 weeks of repeated intake. It was not proven for the second patient that steady-state had been attained. The MQ concentrations 28 days after the last dose intake were for both patients within the same range as observed in healthy volunteers with a similar dose regimen. We do not consider these data as a proof that renal function has no importance for MQ kinetics. Only one patient was suggested to have attained steady-state from only two repeated concentration measurements. A larger sample of subjects with impaired renal function on MQ prophylaxis is required to study the relationship between MQ kinetics and renal function.

The current recommendation for prophylaxis to take 250 mg once weekly and to start this regimen 1 week before entering an area with malaria (i.e. not before the intake of the second dose), results in a total concentration of MQ during the second week that is about half of the concentration at steady-state. A longer loading phase or a shorter initial dosing interval would result in concentrations closer to steady-state at the first time of possible exposure to malaria.

Compared with (–)SR-MQ, (+)RS-MQ has many kinetic advantages. In this study, the half-life was shorter with a more rapid achievement of steady-state, kinetics were not time- or concentration dependent, and no signs of enterohepatic recycling were found. However, it cannot be excluded that such phenomena would occur if (+)RS-MQ alone was given in high enough doses to achieve a prophylactic or therapeutic effect. In addition, the adverse effect profile of this enantiomer at high concentrations needs to be studied.

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Figure 2 Individual observed concentrations of a) the (+)RS-MQ and b) the (-)SR-MQ enantiomers in 10 healthy volunteers during intake of 250 mg racemic mefloquine once weekly. The simulated concentration-time curves are based on the mean population values of the kinetic parameters for each enantiomer in this population.

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