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Divided-dose kinetics of mefloquine in man

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The kinetics of mefloquine was investigated following oral divided-doses in 10 healthy Caucasian volunteers. They received 500 or 750 mg followed by 500 mg 8 h later. Unchanged mefloquine (M) and its carboxylic acid metabolite (MM) were measured in whole blood and plasma for 50 days by h.p.l.c. Maximum blood and plasma M concentrations of 1872 ± 362 ng ml^{-1} (mean ± s.d.) and 1900 ± 434 ng ml^{-1}, respectively, were found within 6–10 h after the second dose. The terminal plasma elimination half-life was 20.1 ± 3.7 days (mean ± s.d.) and the oral clearance was 22.3 ± 6.7 ml h^{-1} kg^{-1} (mean ± s.d.). Plasma concentrations of MM exceeded those of M by 2–3 fold within 2 days. The whole blood concentration of MM was lower than that in plasma but also exceeded the whole blood concentration of M.

Keywords mefloquine pharmacokinetics malaria

Introduction

Mefloquine (M), ((±) erythro-α-(2-piperidyl)-2,8-bis (trifluoromethyl)-4-quinoline methanol), is an effective antimalarial drug in single oral doses against chloroquine-resistant malaria and has been available in France since June 1986 (Desjardins *et al.*, 1979). The main metabolite (MM) of M is 2,8-bis-(trifluoromethyl)-4-quinoline carboxylic acid (Jauch *et al.*, 1980).

M is well tolerated in acute malaria after oral administration with minor side-effects such as nausea, diarrhoea and dizziness (White, 1988). However, vomiting may cause treatment failure because of incomplete drug absorption (Harinasuta *et al.*, 1985). To avoid this, it has been suggested that the total dose of 1250 mg M should be divided into two or three units. M seems to be tolerated better when given according to this regimen. The divided doses are usually administered every 8 h (Danis *et al.*, 1982).

While the pharmacokinetic profile of M has previously been measured in healthy volunteers and patients after single curative oral doses (Desjardins *et al.*, 1979; Schwartz *et al.*, 1982) no information is available on the pharmacokinetic properties of this drug using an 8 h dosage-interval.

Methods

Subjects and drug administration

Six male and four female healthy volunteers, aged between 20 and 38 years and weighing from 45 to 72 kg, were included in this study. None was suffering from malnutrition, gastrointestinal disorders, impaired liver, kidney or cardiovascular function. They took no medication during the week preceding the trial.

The drug was administered as its hydrochloride in the form of tablets (Lariam®; Roche, France) containing an amount equivalent to 250 mg drug base. Each subject received 20 mg M kg^{-1} body-weight, corresponding to a total dose of 1250 mg M for the subjects weighing more than 55 kg and 1000 mg M for those weighing less than 55 kg. The

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first tablets (500 or 750 mg M) were taken at midnight and the following two tablets (500 mg M) the next morning (8 h later) after a light breakfast. The study was approved by the Ethics Committee of the Hôpital Claude Bernard (Paris).

Collection of samples

Samples of blood (5 ml) were collected from each volunteer before administration of the first dose, 8 h later just before the second dose, and then at 10, 12, 14, 16, 18, 20, 24, 32 h and 3, 5, 7, 10, 15, 21, 30, 50 days after the first administration.

Blood was collected in glass Vacutainer® tubes (Becton Dickinson, Sterile EDTA k_3). Within 1 h of collection, 1.5 ml whole blood was transferred to fresh plastic tubes. The Vacutainer® tubes containing 3.5 ml blood were centrifuged for 10 min at 3000 rev min^{-1} and the plasma was transferred to fresh plastic tubes. Whole blood and plasma were stored at −20° C.

Assay methods

M and MM were assayed in plasma and whole blood by h.p.l.c. after liquid–liquid extraction. Since Kapetanovic *et al.* (1983) have reported that M is unstable in the isopropylacetate used for the simultaneous extraction of the drug and its main metabolite, M was extracted with dichloromethane, in which it is stable, and a second extraction was carried out with ethylacetate to measure MM.

To 250 μl blood or plasma were added 250 μl of an aqueous solution of the internal standard (α)-2-pyridyl-2.7-bis (trifluoromethyl)-4-quinolinemethanol (2000 ng ml^{-1}) and 1.0 ml acetonitrile.

After vortex mixing (30 s) and centrifugation at 3000 rev min^{-1} (5 min) the acetonitrile phase was transferred to fresh glass tubes and 1.5 ml of a Tris buffer (25 mM hydroxymethyl-aminoethane; adjusted with 0.1 N HCl to pH 8.2) was added. The mixture was extracted with 6.0 ml dichloromethane on a rotary extractor (30 rev min^{-1}, 15 min). The tubes were centrifuged at 3000 rev min^{-1} (10 min) and the aqueous phase was carefully drawn off and discarded. The dichloromethane phase was evaporated under a gentle stream of nitrogen and the residue containing M was dissolved in the mobile phase and analysed by h.p.l.c.

MM was measured by the following method: 250 μl of the aqueous solution of the internal standard and 1.0 ml of Tris buffer (pH 8.2) were added to 250 μl blood or plasma. This mixture was extracted with 6.0 ml ethylacetate on a rotary extractor (15 min, 60 rev min^{-1}). The organic layer was transferred to fresh glass tubes and evaporated under a gentle stream of nitrogen. The residue was dissolved in the mobile phase and assayed by h.p.l.c.

Chromatography was carried out on a C18 Nucleosil column (15 cm × 0.46 cm i.d.) with a particle size of 3 μm (Société Française de Chromatographie Colonne). The mobile phase consisted of water containing 50% v/v acetonitrile adjusted to pH 4 with triethylamine (2% v/v) and phosphoric acid (3% v/v). The retention times of M and MM were 3.5 and 7 min, respectively.

The limits of detection of M and MM in whole blood or plasma were 50 ng ml^{-1} and 100 ng ml^{-1}, respectively. The inter-assay coefficient of variation for aqueous solutions containing 1000 ng ml^{-1} M was 7.8% and 9.6% for 1500 ng ml^{-1} MM (n = 10). The relative standard deviation for duplicate measurements of plasma or blood drug concentrations was less than 5%. Recovery of M from plasma, in the concentration range of 150–3000 ng ml^{-1}, was between 91 and 99%; recovery of the metabolite, in the range of 500–2000 ng ml^{-1}, was between 62 and 69%. No interference from other antimalarial drugs was expected (Kapetanovic *et al.*, 1983), The intra-assay coefficient of variation was less than 5%.

Pharmacokinetic analysis

The areas under the plasma drug and metabolite concentration-time curves, AUC(0,50) were calculated using the linear trapezoidal rule. The oral clearance of M (CL_{po}) was calculated from the dose divided by AUC(0,50). The terminal elimination half-life of M ($t_{½,z}$) was estimated after fitting a triexponential function to the plasma drug concentration-time data using an iterative curve-fitting programme (Siphar, Simed, Créteil, France). Data were compared using Student's paired t-test.

Results

The mean plasma and whole blood concentration-time profiles of M and MM are shown in Figure 1. The plasma concentrations of M exceeded those in whole blood from the second day of drug administration but the differences were not statistically significant. Over the first 32 h the plasma and whole blood drug concentrations were similar. Maximum plasma and whole blood drug concentrations of 1900 ± 434 ng ml^{-1}

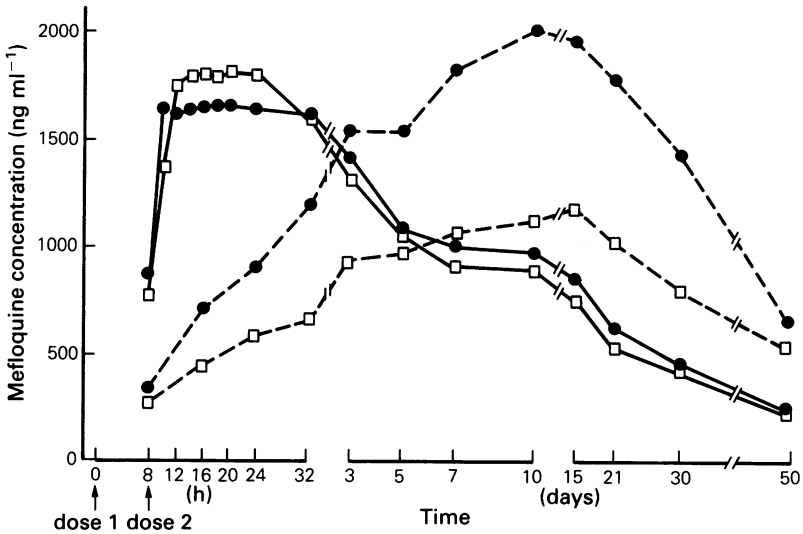

Figure 1 Plasma (•) and whole blood (□) concentrations (mean) of M (——) and MM (---) in healthy volunteers receiving a 20 mg kg^{-1} divided-dose as Lariam® tablets.

(mean ± s.d.) and 1872 ± 362 ng ml^{-1}, respectively, were observed at 24 h. Plasma concentrations of MM exceeded those of M within 2 days, resulting in 2–3 fold higher values with a maximum of 2424 ± 400 ng ml^{-1} (mean ± s.d.). Concentrations of MM in blood were lower than those in plasma but also exceeded those of M with a maximum of 1340 ± 208 ng ml^{-1}.

The differences in the pharmacokinetic parameters derived from plasma or blood concentration profiles were not statistically significant (Table 1).

The drug was well tolerated although three subjects felt slight nausea and dizziness.

Discussion

The pharmacokinetic parameters for M found in this study were within the range of those reported by others using different dosages. Thus, Schwartz *et al.* (1982) and De Souza *et al.* (1987) found mean oral clearances of 18–39 ml min^{-1} kg^{-1} based on plasma drug concentrations and terminal elimination half-lives of 15–33 days.

In the present study, the metabolite was eliminated from plasma at a rate similar to that of M. The AUC ratio MM/M in the plasma was 3.33 in this study compared with 3.0 in the study of De Souza *et al.* (1987). The fact that this ratio was greater than unity signifies that the clearance of MM is less than that of M.

M binds with a high affinity to red cell membranes (Chevli & Fitch, 1982). *In vitro*, red cell drug concentrations are sixty times those in the surrounding buffered saline medium (San George *et al.*, 1984). In earlier studies, red cell drug concentrations were found to be twice as high as corresponding plasma drug concentrations (Schwartz *et al.*, 1982). However, in agreement with a recent study (Karbwang *et al.*, 1987), we found no difference between plasma and whole blood concentrations of M. Presumably, a high plasma binding of M prevents its association with red blood cells. Consequently, either plasma or whole blood may be used to measure drug concentrations in healthy volunteers who are not taking other medication. This could be an advantage in studies where red cell and plasma separation is technically impossible.

It is not clear what plasma concentrations of mefloquine are necessary for the effective treatment of malaria. As M is known to be extensively bound to plasma proteins (98%), any discussion of a concentration effect relationship should refer to the concentration of the unbound drug. Nevertheless, Chanthavanich *et al.* (1985) reported that a plasma drug concentration of 500 ng ml^{-1} achieved during the first days of treatment in Thai patients (15.6 mg kg^{-1}) was associated with a cure rate exceeding 95%. Boudreau *et al.* (1986) found that plasma M concentrations greater than 1000 ng ml^{-1} on the second day of treatment were associated with successful treatment. In the present study maximum plasma and

Table 1 Details of volunteers and pharmacokinetic parameters derived from blood and plasma drug concentration-time profiles of mefloquine (M) and its main metabolite (MM)

					M blood					*M plasma*					*MM blood*			*MM plasma*		
Patient	*Sex*	*Age (years)*	*Weight (kg)*	*Dose (mg)*	t_{max} *(h)*	C_{max} *(ng ml^{-1})*	$t_{1/2z}$ *(days)*	*AUC (μg ml^{-1} day)*	CL_{po} *(ml h^{-1} kg^{-1})*	t_{max} *(h)*	C_{max} *(ng ml^{-1})*	$t_{1/2z}$ *(days)*	*AUC (μg ml^{-1} day)*	CL_{po} *(ml h^{-1} kg^{-1})*	t_{max} *(days)*	C_{max} *(ng ml^{-1})*	*Ratio of area MM/M*	t_{max} *(days)*	C_{max} *(ng ml^{-1})*	*Ratio of area MM/M*
1	M	29	67	1250	16	1650	15.8	29.2	26.7	—	—	—	—	—	10	1490	2.7	—	—	—
2	M	26	55	1250	20	2237	12.3	28.5	33.3	18	1966	22.6	45.7	20.7	15	1192	3.4	15	3041	3.9
3	M	28	72	1250	24	2222	21.9	38.4	18.9	20	2400	17.1	38.4	18.9	—	—	—	21	1907	4.2
4	M	30	63	1250	24	1850	20.2	44.1	18.7	20	2500	14.1	45.8	18.0	10	1470	2.0	5	2190	2.7
5	F	29	50	1000	14	1440	46.5	47.7	17.5	16	1580	24.0	41.4	20.1	15	1156	1.3	15	2754	3.4
6	F	20	48	1000	18	2035	24.5	43.8	19.8	24	2160	23.6	51.7	16.7	—	—	—	21	1931	1.3
7	F	32	45	1250	20	2350	13.6	35.1	32.9	18	1800	16.0	35.4	32.7	15	1200	1.7	10	2222	3.3
8	F	34	50	1000	16	1250	17.1	21.0	39.6	16	1070	18.9	24.5	34.1	7	1157	2.5	10	2340	4.2
9	M	38	60	1250	12	1700	23.1	34.8	24.9	18	1750	21.6	36.2	24.0	15	1325	4.3	7	2650	3.9
10	M	25	58	1125	24	1990	18.6	49.5	16.3	24	1870	23.2	51.0	15.9	21	1733	1.7	21	2786	3.0
Mean ± s.d.					18.8 ±4.3	1872 ±326.7	21.4 ±9.7	37.2 ±9.2	24.9 ±8.1	19.3 ±3.0	1900 ±434	20.1 ±3.7	41.4 ±8.6	22.3 ±6.7	13.5 ±4.4	1340 ±208	2.5 ±1.0	13.9 ±6.2	2424 ±400	3.3 ±0.9

blood drug concentrations exceeding 1000 ng ml^{-1} were obtained within 10 h after administration of the first dose. As the volume of distribution of mefloquine is smaller in malarial patients than in healthy volunteers, one may expect higher blood and plasma drug concentrations in the former group (Looareesuwan *et al.*, 1987). Consequently, effective concentrations in malaria patients should be attained within 10 h following the present regimen. In order to destroy all of the parasites, it is necessary to maintain an effective concentration for two to three times the life cycle of the parasite, that is 4–6 days. In this study concentrations greater than 1000 ng ml^{-1} persisted on the fifth day of administration, with blood drug concentrations falling below the effective level on the seventh day.

As plasma metabolite concentrations exceeded those of M, the question arises as to whether the metabolite contributes to therapeutic response. However, this contribution should be negligible since the respective IC_{50} values of MM and M were found to be 1000, > 85000, > 85000 nmol l^{-1} and 8.5, 14.5, 7.4 nmol l^{-1} using three *falciparum* strains (unpublished data). On the other hand, MM may contribute to the side-effects of treatment (Rouveix *et al.*, 1989).

Based upon the present findings, a computer simulation of various dosage schedules showed that when the dosing interval is maintained between 8 to 24 h, maximum and effective concentrations should be reached at 12 h and maintained for 30–60 h.

Thus, it may be possible to reduce the problems of patient non-compliance due to side-effects and low plasma mefloquine concentrations due to vomiting caused by doses exceeding 750 mg (Tin *et al.*, 1987) by giving mefloquine in a divided oral dose at intervals of 8 to 24 h.

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