Disposition of the enantiomers of hydroxychloroquine in patients with rheumatoid arthritis following multiple doses of the racemate

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In eight patients with rheumatoid arthritis receiving racemic hydroxychloroquine, blood and urine concentrations of the enantiomers of hydroxychloroquine and its major metabolites were measured each month over the first 6 months of therapy. Plasma concentrations of hydroxychloroquine enantiomers were measured in five of these patients. In all patients, the blood concentration of (R)-hydroxychloroquine exceeded that of the (S)-enantiomer, the mean (R)/(S) ratio being 2.2 (range 1.6–2.9). A similar excess of (R)-hydroxychloroquine was found in the plasma, the mean (R)/(S) ratio being 1.6 (range 1.2–1.9). The mean enantiomer blood concentration ratio (R)/(S) for the metabolite desethylhydroxychloroquine was 0.45 (range 0.34–0.58) and for desethylchloroquine it was 0.56 (range 0.35–0.86) suggesting stereoselective metabolism of hydroxychloroquine. (S)-hydroxychloroquine had a mean ($\pm$ s.d.) renal clearance from blood of 41 ± 11 ml min⁻¹, approximately twice that of (R)-hydroxychloroquine. The predicted unbound renal clearance was also higher for (S)-hydroxychloroquine. The clinical implications of enantioselective disposition of hydroxychloroquine are currently not known.

Keywords hydroxychloroquine pharmacokinetics enantiomers steady-state

Introduction

Hydroxychloroquine was developed originally as an antimalarial agent but is more often used as a slow-acting anti-rheumatic drug in the management of connective tissue disorders. It is administered as a racemate but no information is available about the possibilities of stereoselective disposition and activity.

The aim of this study was to characterise the disposition of the enantiomers of hydroxychloroquine and its metabolites following multiple dosing with rac-hydroxychloroquine in patients with rheumatoid arthritis.

Methods

Subjects

Eight patients with rheumatoid arthritis according to A.R.A. criteria and who were considered by their rheumatologist as suitable to commence hydroxy-

chloroquine therapy were enrolled into the study. The mean age of the patients was 54 years (range; 40–72) and the mean weight was 72 kg (range; 56–89). Patients 4 and 8 were male. All patients were taking non-steroidal anti-inflammatory drugs, but none was taking any other slow-acting anti-rheumatic drugs. Patients were also receiving a range of other medications for the management of co-existing diseases. Patient 1 was receiving medroxyprogesterone, patient 2, diazepam, patient 3, hydrochlorthiazide/amiloride and conjugated oestrogens, patient 4, cimetidine, and patient 6 was receiving thyroxine. Patient 3 was a regular cigarette smoker (20–40 per day).

The study had the approval of the Research Ethics Committee of St Vincent's Hospital and informed consent was obtained from each patient in writing. All patients underwent full medical, haematological and biochemical examination and had full ophthalmological review and audiological tests before commencing therapy.

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Dosage

Patients 2, 4, 6 and 7 received an oral daily dose of 155 mg rac-hydroxychloroquine (one tablet of PlaquenilTM, 200 mg rac-hydroxychloroquine sulphate), equivalent to 77.5 mg of each enantiomer. Patients 1, 3, 5 and 8 received 310 mg rac-hydroxychloroquine (two tablets of PlaquenilTM) daily, equivalent to 155 mg of each enantiomer. These doses were assigned randomly as part of a larger, double-blind study investigating the pharmacodynamics of hydroxychloroquine.

Sample collection and analysis

Blood and urine samples were collected from each patient during a clinic visit at the end of each month for the first 6 months of therapy with hydroxychloroquine. The blood samples were collected approximately 24 h after the previous hydroxychloroquine dose into silanized glass vacutubes (with 125 i.u. heparin added per 10 ml). The time of blood sampling and the last dose were recorded. The volume and the time over which the bladder was filled was recorded for each urine sample and an aliquot retained for analysis. Plasma samples were available from patients 3, 4, 5, 6 and 7 at the same times. Plasma was separated from cells as described previously [1], by centrifugation at 1200 g for 20 min within 1 h of sample collection.

All samples were stored in polypropylene tubes after analysis of racemic drug and the major metabolites, desethylhydroxychloroquine and desethylchloroquine [2] and samples were re-frozen at -18°C until stereoselective assay by the method of McLachlan *et al.* [3].

Data treatment

The urinary excretion rate of each enantiomer was calculated as its urine concentration multiplied by the void volume divided by the time of bladder filling. The renal clearance of each enantiomer was calculated by dividing its urinary excretion rate by its blood or plasma concentration. For five patients the unbound renal clearance of the individual enantiomers was calculated using estimates of the free plasma concentrations of (R)- and (S)-hydroxychloroquine based on data for the *in*

vitro protein binding of hydroxychloroquine enantiomers [4].

Parameters are expressed as mean $\pm$ s.d.

Results

Blood and plasma concentrations of the enantiomers of hydroxychloroquine and blood concentrations of the enantiomers of the two major metabolites (desethylchloroquine and desethylhydroxychloroquine) over the first 6 months of hydroxychloroquine therapy in one patient are shown in Figure 1.

Table 1 lists the blood and plasma concentration ratios of the enantiomers of hydroxychloroquine and the blood concentration ratios of the enantiomers of the major metabolites in the patients. The concentrations of the enantiomers of the metabolites in plasma were below the assay sensitivity. The concentration of (R)-hydroxychloroquine exceeded that of the (S)-enantiomer in the blood and plasma of all the patients at each sampling time. The blood concentrations of the (S)-enantiomers of both the major metabolites, desethylchloroquine and desethylhydroxychloroquine, exceeded those of the (R)-enantiomers. Concentrations of the enantiomers of bisdesethylchloroquine were below the limits of determination of the assay.

Mean renal clearances of the enantiomers of hydroxychloroquine from blood over the first six months of therapy were $41 \pm 11 \text{ ml min}^{-1}$ (range: 21–55) for the (S)-isomer and $19 \pm 9 \text{ ml min}^{-1}$ (range: 10–39) for the (R)-isomer. Mean renal clearances from plasma were $277 \pm 349 \text{ ml min}^{-1}$ (range: 22–890) for the (S)-isomer and $199 \pm 290 \text{ ml min}^{-1}$ (range: 11–715) for the (R)-isomer.

The mean ratios ((R)/(S)) of the renal clearance of the enantiomers of hydroxychloroquine calculated using blood and plasma concentration data were 0.45 ± 0.14 (range 0.30–0.75) and 0.60 ± 0.15 (range 0.44–0.81), respectively.

Estimates of mean unbound renal clearance of the enantiomers of hydroxychloroquine in the five patients for whom plasma data were available were $316 \pm 460 \text{ ml min}^{-1}$ (range: 17–1133) for the (R)- enantiomer and $771 \pm 972 \text{ ml min}^{-1}$ (range: 58–2480) for the (S)-isomer.

Table 1 Mean ratios ((R)/(S)) of the concentrations of the enantiomers of hydroxychloroquine and metabolites over the initial 6 months of therapy in patients with rheumatoid arthritis

Patient	Hydroxychloroquine		Desethyl- hydroxychloroquine	Desethyl- chloroquine
	Blood	Plasma	Blood	Blood
1	1.63		0.38	0.35
2	1.93		0.50	0.49
3	2.60	1.67	0.34	0.43
4	1.80	1.82	0.44	0.50
5	2.84	1.46	0.39	0.71
6	2.11	1.20	0.46	0.86
7	2.93	1.86	0.58	0.78
8	1.97		0.48	0.39
Mean	2.23	1.60	0.45	0.56
$\pm$ s.d.	0.49	0.28	0.08	0.19

N.B. Plasma samples were unavailable for patients 1,2 and 8.

Figure 1 Blood (—) and plasma (---) concentrations of the enantiomers of hydroxychloroquine (HCQ (R) —■—, (S) —●—) and blood concentrations of the enantiomers of desethylchloroquine (CQM (R) —■—, (S) —▲—) and desethylhydroxychloroquine (HCQM (R) —■—, (S) —▲—) over the first 6 months of hydroxychloroquine therapy in a patient with rheumatoid arthritis.

Renal clearance of metabolites was also stereoselective. For most patients renal clearance of the (S)-enantiomers of the metabolites exceeded that of the (R)-enantiomers. The mean renal clearance of desethylhydroxychloroquine from blood was $3.2 \pm 1.6 \text{ ml min}^{-1}$ (range 1.4–6.1) for the (R)-enantiomer and $5.4 \pm 2.3 \text{ ml min}^{-1}$ (range 2.5–9.8) for the (S)-enantiomer. For desethylchloroquine, the mean renal clearance from blood was $5.3 \pm 2.1 \text{ ml min}^{-1}$ (range 2.2–7.4) for the (R)-isomer and $7.5 \pm 3.8 \text{ ml min}^{-1}$ (range 4.0–16.4) for the (S)-enantiomer. The mean ratios ((R)/(S)) of the renal clearance of the enantiomers of desethylhydroxychloroquine and desethylchloroquine calculated from blood data were 0.64 ± 0.26 (range 0.15–100) and 0.78 ± 0.28 (range 0.45–1.17), respectively.

Discussion

The higher blood and plasma concentrations of (R)-hydroxychloroquine compared with the (S)-enantiomer observed in all patients confirm the operation of a stereoselective process or processes in the disposition of hydroxychloroquine. These processes could include chiral inversion, stereoselective absorption, distribution (for example, plasma protein binding or red cell uptake), metabolism and/or stereoselective renal excretion. There was almost a two-fold variation in the ratio of (R)- to (S)-hydroxychloroquine blood concentrations (1.6 to

2.9) between the patients, suggesting significant inter-patient variability in the stereoselective processes. Similar observations of enantioselective disposition have been reported for the structurally-related drug chloroquine, the concentrations of (R)-chloroquine being about 1.3 times those of the (S)-enantiomer in plasma and 1.8 times those of the (S)-enantiomer in the blood of four patients with rheumatoid arthritis [5].

Considering the stability of groups in the vicinity of the chiral centre of hydroxychloroquine, the possibility of chiral inversion for this molecule seems remote. (R)- and (S)-chloroquine have been incubated separately with human liver microsomes *in vitro* and produced optically pure metabolite enantiomers subsequently used as standards in an assay [6].

The data suggest that the *N*-deethylation of hydroxychloroquine is stereoselective, (S)-hydroxychloroquine being more susceptible than the (R)-enantiomer of hydroxychloroquine. The plasma and blood concentrations of (S)-desethylchloroquine and (S)-desethylhydroxychloroquine exceeded those of the (R)-metabolites in all of the patients studied. This finding is consistent with the data for chloroquine metabolites [5, 6].

Renal clearance also appears to be stereoselective for hydroxychloroquine even when corrected for plasma protein binding. These data are in broad agreement with those found for the enantiomers of chloroquine and desethylchloroquine [5, 6].

The estimated unbound renal clearance of (R)-hydroxychloroquine in patients 3, 6 and 7 (aged 58, 44 and 48 years) approximated the expected glomerular filtration rate of 130 ml min^{-1} . The estimated unbound renal clearance of the (S)-enantiomer in the same patients was similar to the expected renal plasma flow rate of 650 ml min^{-1} . These data suggest that (S)-hydroxychloroquine underwent net active secretion into the renal tubules, while the (R)-antipode was filtered but not secreted, or re-absorbed to the same extent as secreted.

The unbound renal clearance data calculated for patients 4 and 6 appear to be artefactually low and high, respectively, most probably due to difficulties with the accurate and reproducible measurement of plasma concentrations due to technical difficulties with separation of plasma [1, 7]. Although absolute plasma concentration values and renal clearance values calculated from these data are probably not to be relied upon, it is still valuable to examine enantiomeric ratios to gain some insight into stereospecific disposition.

Renal clearance is unlikely to explain all of the stereoselective differences in the blood and plasma drug concentrations as renal clearance accounts for only a small percentage of total clearance. Following intravenous administration of racemic hydroxychloroquine, renal clearance of racemate (mean 20 ml min^{-1} from blood) has been estimated to account for approximately 20% of total clearance (mean 96 ml min^{-1} from blood) [1].

No information is available about the relative efficacy and toxicity of the enantiomers of hydroxychloroquine. Studies have suggested that (S)-chloroquine is more effective than (R)-chloroquine in the treatment of certain strains of malaria [8, 9]. Limited toxicity data in mice indicate that (R)-chloroquine is the more toxic

isomer as it has a lower LD₅₀ than the (S)-enantiomer [8]. It is not possible to extrapolate these data to the chronic use of the antimalarials in the therapy of rheumatoid arthritis.

Enantioselective elimination of hydroxychloroquine, both metabolic and renal, has been demonstrated in patients receiving chronic dosing with rac-hydroxychloroquine. The stereoselectivity of these processes shows interpatient variability which may contribute to

variability in response and the inability, to date, to demonstrate a useful relationship between concentrations of racemic drug and effect.

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