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Enantioselective disposition of hydroxychloroquine after a single oral dose of the racemate to healthy subjects

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1 Stereoselectivity in the disposition of hydroxychloroquine was investigated in 23 healthy males following a single oral dose of 200 mg racemic HCQ (*rac*-HCQ) sulphate. Total concentrations (R+S) and R/S ratios of HCQ and its metabolites were measured by stereoselective h.p.l.c.

2 HCQ was detected in whole blood and urine, up to 91 and 85 days after dosing, respectively. Metabolites could not be detected in whole blood while in urine detectable concentrations were still present after 85 days. The blood concentrations of HCQ enantiomers were measurable until 168 h post-dose.

3 R(−)-HCQ accounted for 62 ± 3% (mean ± s.d.) of the AUC of *rac*-HCQ AUC. The elimination half-life of S(+)-HCQ (457 ± 122 h) was significantly shorter than that of R(−)-HCQ (526 ± 140 h), partly due to its faster urinary excretion and hepatic metabolism. Its renal clearance was twice that of R(−)-HCQ (4.61 ± 4.01 *vs* 1.79 ± 1.30 l h^{-1}), and metabolites derived from the S-isomer represented 80–90% of the urinary recovery of the dose.

4 Over 85 days, 4.4 ± 2.9 and 3.3 ± 1.8% of the dose was recovered in urine as unchanged S(+)-HCQ and R(−)-HCQ, respectively. For the first 2 weeks, S(+)-HCQ excretion rate clearly surpassed that of R(−)-HCQ whereas afterwards the inverse was observed. However, since the first 2 weeks account for 95% of *rac*-HCQ renal excretion, the total urinary excretion of S(+)-HCQ clearly surpassed that of R(−)-HCQ.

5 In urine, the R/S ratios of desethylhydroxychloroquine (DHCQ) were stable while those of desethylchloroquine (DCQ) increased over time. Since both desethylations display a different enantioselectivity, different enzymes appear to be responsible for HCQ metabolism into DCQ and DHCQ.

Keywords hydroxychloroquine enantiomers whole blood urine pharmacokinetics

Introduction

Hydroxychloroquine (HCQ) is an antimalarial agent, which is also an effective treatment against rheumatoid arthritis for subjects with persistent or progressive disease [1]. It is administered as a racemic mixture [*rac*-HCQ] of two isomers, R(−)-HCQ and S(+)-HCQ. Hepatic metabolism generates three metabolites, desethylchloroquine (DCQ), desethylhydroxychloroquine (DHCQ) and bisdesethylchloroquine (BDCQ), which are also chiral molecules [2]. Documentation of the pharmacokinetics of each enantiomer might provide useful insight with regard to concentration-effect relationships.

Preliminary data from a cross-sectional study of HCQ pharmacodynamics failed to associate HCQ toxicity or efficacy with a single enantiomer: arthritis patients suffering from less severe disease had significantly higher steady-state concentrations of both enantiomers [3]. However, since patients were on

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different concomitant medications (e.g. analgesics, methotrexate, sulphasalazine), pharmacodynamic relationships remain to be determined prospectively in a more homogenous population [4].

Enantioselectivity in the pharmacokinetics of HCQ has been previously studied in eight subjects on chronic *rac*-HCQ treatment [5]. Because the elimination half-life of HCQ is about 40 days [6], monthly blood, plasma and urine concentrations of R(−) and S(+)-HCQ were measured over the first 6 months of therapy (six samples). In blood and plasma, concentrations of R(−)-HCQ exceeded those of S(+)-HCQ, whereas concentrations of the S(+)-enantiomers of the metabolites were higher than those of the R(−)-forms. At steady-state, the renal clearance of S(+)-HCQ was double that of R(−)-HCQ.

McLachlan *et al.* [7] investigated the absorption and early distribution of HCQ enantiomers following a single dose of *rac*-HCQ. Absorption was not stereoselective and comparable fractions of each enantiomer were absorbed (74% of the dose for the R compared with 77% for the S). Since blood samples were drawn for only 32 h after dosing, no information is available regarding the later distribution and elimination of the individual enantiomers. The present study describes the blood and urine profiles of the enantiomers of HCQ, DCQ and DHCQ during the first 3 months following a single oral dose of *rac*-HCQ.

Methods

Protocol

The study was carried out at the PRACS Institute (Fargo, ND, USA) under medical supervision, after Ethics Committee approval. Twenty-four subjects gave written informed consent to participate in the study. All were male, non-smoking adults of standard height (mean ± s.d., 181 ± 7 cm) and weight (80 ± 8 kg), aged from 20 to 36 years (25 ± 4). They were all within 20% of their ideal body weight (101 ± 9%). Complete physical and ophthalmological examination, as well as routine haematological and biochemical tests were performed to ensure that the subjects were medically fit.

After an overnight fast, each subject received a single oral dose of HCQ sulphate (one 200 mg Plaquenil® tablet). Blood was collected from a peripheral vein into EDTA Vacutainer™ tubes. Samples (3 ml) were obtained before dosing and thereafter at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120 h and 7, 11, 14, 18, 21, 25, 28, 32, 35, 42, 49, 56, 63, 70, 77, 84 and 91 days. Vacutainer™ tubes were inverted twice, then blood was transferred into a polypropylene tube and stored at −20° C until analysis.

A total of 18 urine samples were collected in polypropylene containers. On day 1, urine was collected over the hour before dosing, then after dosing from 0–4, 4–8, 8–12, 12–16 and 16–24 h, followed by 24 h collections on days 2, 3, 4, 5, 8, 12, 15, 29, 43, 57, 71 and 85. Samples were mixed gently within the container, measured by weight, and 20 ml aliquots were placed into polypropylene tubes and frozen at −20° C until analysis.

Analytical methods

Whole blood Whole blood samples were assayed by h.p.l.c. with u.v. detection according to the slightly modified method of Iredale & Wainer [8]. Since no metabolites were detected, extracted samples were injected directly into the chiral system, without the first-step achiral separation. In brief, samples (1 ml) were diluted 1 : 1 with water, alkalinized, spiked with the internal standard (chloroquine), and extracted twice with diethyl ether. The organic phase was back-extracted with hydrochloric acid and the aqueous phase directly injected into the α_1-acid glycoprotein chiral stationary phase. The lower limit of determination of the assay was 5 ng ml^{-1} of each HCQ enantiomer, with 96 to 98% accuracy. The intra- and inter-day coefficients of variation were 2.9 and 4.9% respectively, for higher concentrations and 10.4 and 12.0% respectively, for lower concentrations.

Urine Since urine contained significant amounts of metabolites, samples had to be analyzed by sequential achiral-chiral analysis [9]. HCQ and its three metabolites were first separated on a cyano-bonded phase. For all analytes, the inter-day coefficients of variations ranged from 2.2 to 3.7%, the accuracy from 85 to 104%, and the lowest limit of quantitation was 5 ng ml^{-1}. The eluents containing HCQ, DCQ and DHCQ were collected, alkalinized, and extracted with diethyl ether before injection onto the α_1-acid glycoprotein chiral stationary phase to determine the enantiomeric ratios. For HCQ and its metabolites, this system can detect down to 5 ng ml^{-1} of each enantiomer, with inter-day coefficients of variation of 1.8 to 3.5%.

Pharmacokinetic analysis

Whole blood data Values of C_{max} and t_{max} were obtained directly from the data. The terminal elimination rate constant (λ_z) was determined by fitting the blood concentration *vs* time data to a mamillary three-compartment model with first-order oral absorption. Generalized least squares analysis was performed using ADAPT II [10]. The terminal elimination half-life ($t_{1/2,z}$) was calculated from $0.693/\lambda_z$. All other parameters were estimated using non-compartmental techniques [11]. The area under the blood HCQ enantiomers concentration *vs* time curve (AUC) and the area under the first moment curve (AUMC) were estimated using the trapezoidal rule. The portion of the AUC from the last time point (t) to infinity (inf) was calculated from the last measurable concentration (C_t) divided by λ_z. The portion of the AUMC from t to infinity (inf) was calculated from $[(t \times C_t)/\lambda_z] + [C_t/\lambda_z^2]$. The mean transit time (MTT) was estimated by the ratio AUMC/AUC. The total oral clearance

(CL/*F*) was calculated by dividing the dose with the AUC (*F* being the unknown oral bioavailability of the drug). Since HCQ absorption time is negligible compared with its prolonged elimination [6], the mean residence time (MRT) approximates the MTT. Therefore, the apparent volume of distribution at steady-state (V_{ss}/F) could be obtained by the product of the CL/*F* × MTT, i.e. dose × AUMC/AUC^2.

Urinary data The urinary recoveries of HCQ and its metabolites (*Ae*) were calculated up to 86 days after dosing, the actual times depending on the detectability of the analyte in urine. The percentage of the dose excreted in urine as unchanged HCQ, DCQ or DHCQ was calculated from the molar ratio of *Ae*/Dose.

The half-lives of *rac*-HCQ, R(−)-HCQ and S(+)-HCQ were calculated from the slope of the terminal linear portion of the log urinary excretion rate (Ae_{0-t}/t) *vs* time (midpoint of the collection time) curves using the last 6 data points. Renal clearance (CL_R) was calculated from urinary recovery (Ae_{0-t}) divided by AUC(0,*t*) where *t* was the last time at which the drug could be measured in both whole blood and urine.

Statistical analysis Results are presented as mean ± standard deviation (s.d.). The 95% confidence intervals were also calculated. Pharmacokinetic parameters derived from blood R(−)-HCQ and S(+)-HCQ concentrations were compared using Student's *t*-test for paired data. To define whether R/S ratios of CL_R changed over time, relationships with time were investigated using linear regression. The level of statistical significance was set at 0.05.

Results

Incomplete data were obtained for one subject and these were excluded from the analysis. In another subject, only unresolved HCQ concentrations were measured. Mean whole blood HCQ concentrations are shown in Figure 1a. C_{max} values of unresolved drug ranged from 54 to 206 ng ml^{-1} and were reached between 2 and 6 h after dosing (Table 1). HCQ was detectable in the whole blood of all subjects up to 49 days. At 84 and 91 days, low concentrations (approximating the limit of quantitation) were detected in only three subjects and one subject, respectively. On average HCQ could be measured up to 64 days. No metabolites were detected in whole blood.

The area under the blood HCQ concentration-time curve until the last detectable concentration (at time *t*) (12 312 ± 3471 ng ml^{-1} h, mean ± s.d.) accounted for 86 ± 5% (76 to 93%) of the AUC extrapolated until infinity (AUC, 14 177 ± 3475 ng ml^{-1} h). The terminal elimination half-life ($t_{1/2,z}$) was 563 ± 144 h (23 ± 6 days). The mean transit time (MTT) was estimated at 626 ± 249 h (26 ± 10 days). HCQ apparent

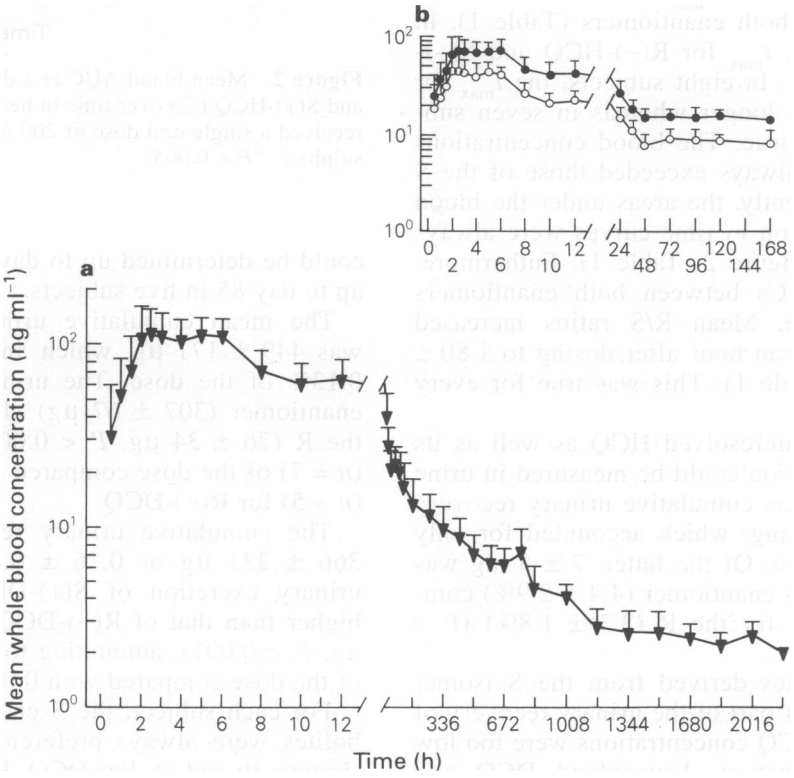

Figure 1 Mean whole blood concentration (± s.d.) *vs* time profiles of *rac*-HCQ (▼) in 23 healthy subjects who received a single oral dose of 200 mg of *rac*-HCQ sulphate (a). Mean whole blood concentration *vs* time profiles of R(−)-HCQ (●) and S(+)-HCQ (○) are shown on the upper graph (b).

Table 1 Mean ± s.d. (95% confidence interval) pharmacokinetic parameters of R- and S-HCQ following a single oral dose of 200 mg of *rac*-HCQ sulphate in 23 healthy volunteers

Parameter	rac-*HCQ*	*R(−)-HCQ*	*S(+)-HCQ*	*R/S ratio*	P (*R* vs *S*)
Blood data					
C_{max} (ng ml^{-1})	129 ± 39 (114–145)	84 ± 22 (74–93)	52 ± 14 (46–58)	1.61 ± 0.18 (1.53–1.69)	<0.001
t_{max} (h)*	3.4 (2.8–3.9)	3.0 (2.9–4.1)	2.5 (2.7–3.9)	1.13 ± 0.47 (0.93–1.33)	NS
AUC(0,168h) (ng ml^{-1} h)	6382 ± 517 (6170–6594)	3948 ± 351 (3805–4091)	2208 ± 343 (2069–2347)	1.80 ± 0.17 (1.74–1.86)	0.003
Urine data					
$t_{1/2,z}$(h)	492 ± 129 (440–545)	526 ± 140 (468–583)	457 ± 122 (407–507)	1.16 ± 0.10 (1.11–1.19)	<0.001
CL_R (l h^{-1})	2.74 ± 1.63 (1.53–2.84)	1.79 ± 1.30 (0.83–2.76)	4.61 ± 4.01 (1.64–7.56)	0.41 ± 0.04 (0.38–0.44)	<0.001

*Median.

volume of distribution (V_{ss}/F) was very large, being 7760 ± 4480 l. The total oral clearance (CL/*F*) was 11.6 ± 2.7 l h^{-1} (193 ± 45 ml min^{-1}).

Whole blood concentrations of R(−)-HCQ and S(+)-HCQ are shown in Figure 1b. While R and S concentrations could be measured up to 72 h in most subjects, only three showed detectable enantiomer concentrations after 168 h. C_{max} values were consistently higher for the R enantiomer and accounted for 63 ± 5% of unresolved HCQ peak concentrations (Table 1). Although median t_{max} values did not differ significantly between both enantiomers (Table 1), in 15 out of 23 subjects, t_{max} for R(−)-HCQ and S(+)-HCQ did not coincide. In eight subjects, the t_{max} for the R enantiomer was longer whereas in seven subjects the inverse was true. The blood concentrations of the R enantiomer always exceeded those of the S (Figure 1b). Consequently, the areas under the blood R(−)-HCQ concentration *vs* time curves were always significantly larger (Figure 2, Table 1). Futhermore, the difference in AUCs between both enantiomers augmented with time. Mean R/S ratios increased from 1.41 ± 0.26 half an hour after dosing to 1.80 ± 0.17 after 7 days (Table 1). This was true for every subject.

In all 23 subjects, unresolved HCQ as well as its enantiomeric composition could be measured in urine up to day 85. The mean cumulative urinary recovery of HCQ was 12 ± 7 mg, which accounted for only 7.7 ± 4.8% of the dose. Of the latter, 7 ± 5 mg was accounted for by the S enantiomer (4.4 ± 2.9%) compared with 5 ± 3 mg for the R (3.3 ± 1.8%) ($P < 0.001$).

Similarly, metabolites derived from the S isomer accounted for the most part of the urinary recovery of DCQ and DHCQ. BDCQ concentrations were too low for accurate determination. Unresolved DCQ and DHCQ urine levels could be measured up to day 57 in all subjects, and up to day 85 in 17 and 23 subjects, respectively. Their enantiomeric composition could be determined up to day 57 in 15 subjects, and up to day 85 in five subjects.

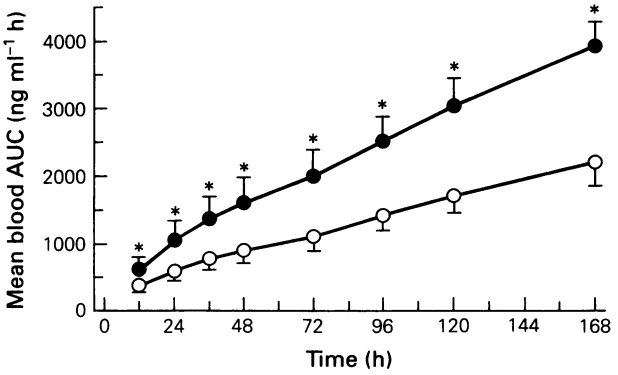

Figure 2 Mean blood AUC (± s.d.) of R(−)-HCQ (●) and S(+)-HCQ (○) over time in healthy subjects who received a single oral dose of 200 mg of *rac*-HCQ sulphate. *$P < 0.005$.

The mean cumulative urinary recovery of DCQ was 449 ± 171 μg, which amounted to only 0.33 ± 0.13% of the dose. The urinary excretion of the S enantiomer (307 ± 67 μg) clearly surpassed that of the R (76 ± 34 μg, $P < 0.001$), with 0.23 ± 0.05% ($n = 7$) of the dose compared with only 0.06 ± 0.03% ($n = 5$) for R(−)-DCQ.

The cumulative urinary recovery of DHCQ was 366 ± 231 μg or 0.26 ± 0.16% of the dose. The urinary excretion of S(+)-DHCQ was significantly higher than that of R(−)-DCQ (359 ± 247 *vs* 49 ± 7 μg, $P < 0.001$), amounting to 0.25 ± 0.17% ($n = 13$) of the dose compared with 0.03 ± 0.01% ($n = 5$).

For each subject, the S enantiomers of both metabolites were always preferentially excreted in urine (Figure 3b and c). For DCQ, R:S percentages in urine changed from approximately 20:80 in the first few days after dosing to 30:70 after 85 days (Figure 3b). The R/S ratios were found to significantly increase

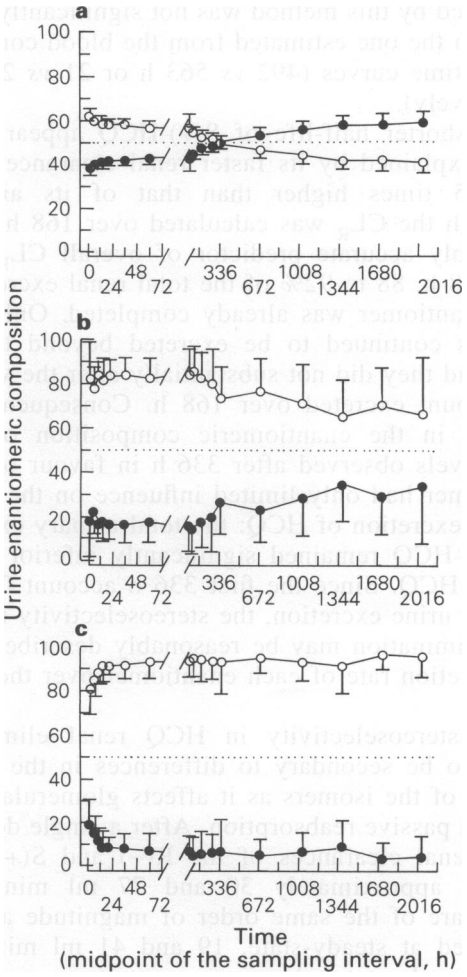

Figure 3 Mean enantiomeric composition (± s.d.) of HCQ (a), DCQ (b) and DHCQ (c) urine concentrations in 23 healthy subjects who received a single oral dose of 200 mg of *rac*-HCQ sulphate. ● R(−)-enantiomer; ○ S(+)-enantiomer.

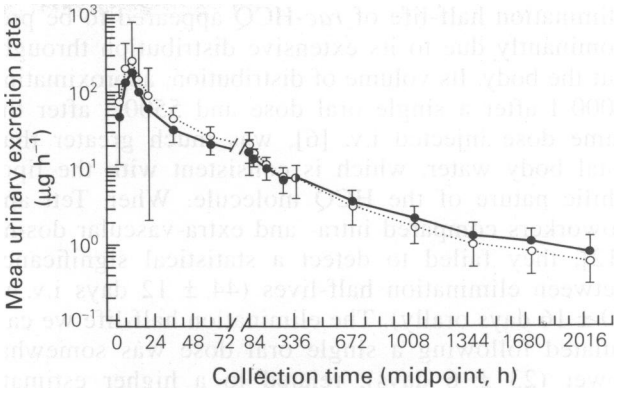

Figure 4 Semilogarithmic plot of the rate of excretion (mean ± s.d.) of R(−)-HCQ (●) and S(+)-HCQ (○) against the midpoint time of urine collection in 23 healthy subjects who received a single oral dose of 200 mg of *rac*-HCQ sulphate.

with time ($P < 0.001$). This finding was observed for every subject. In contrast, DHCQ enantiomeric composition in urine was stable, with R:S proportions of approximately 10:90 (Figure 3c). The R/S ratios were not increasing with time.

In urine, both metabolites accounted for the elimination of less than 1% of the dose. Combining the urinary recoveries of the parent compound with those of the metabolites, approximately 10% of the dose could be accounted for by urinary excretion. The renal clearance of *rac*-HCQ accounted for 24% of total clearance, leading to a non-renal clearance of approximately 8.86 l h^{-1} or 76% of the total clearance.

The time-course of the urinary excretion rate of HCQ enantiomer is presented in Figure 4. For both R(−) and S(+)-HCQ, the excretion rate was maximal 8 to 12 h after dosing. Before 336 h, the excretion rate of S(+)-HCQ was significantly higher than that of R(−)-HCQ ($P < 0.001$). However, after 336 h, there was a complete reversal in the enantiomeric composition of urine HCQ concentrations in favour of the R enantiomer (Figure 4a). R(−)-HCQ concentrations gradually increased from 40 to 60% of unresolved HCQ urine levels. Until the end of the collection period on day 85, the urinary excretion rate of R(−)-HCQ was superior to that of S(+)-HCQ (Figure 4, $P < 0.001$). This finding was observed for all subjects.

The elimination half-lives calculated from the slope of the terminal portion of the urinary excretion rate *vs* time curves were significantly different between both enantiomers (Figure 4). S(+)-HCQ had a shorter half-life of 457 ± 122 h, or 19 days, compared with 526 ± 140 h, or 22 days, for R(−)-HCQ (Table 1).

Since the enantiomeric composition of blood HCQ concentrations was determined up to 168 h, the renal clearance was calculated from blood and urine data over the first 7 days after dosing. The CL_R of S(+)-HCQ was approximately 2.5 times than of R(−)-HCQ, with 4.61 l h^{-1} or 76.8 ml min^{-1} compared with 1.79 l h^{-1} or 29.9 ml min^{-1} (Table 1).

Discussion

Documentation of any stereoselectivity in the pharmacokinetics of HCQ after a single dose has been restricted by the lack of a specific and sensitive assay which can be used in a clinical setting. Using a recently published method which measures HCQ and its metabolites down to 5–10 ng ml^{-1}, we were able to closely follow blood and urine levels up to 85 days after a single oral dose [8, 9]. For *rac*-HCQ pharmacokinetics, our results are within the range of those reported previously after i.v. and oral administration of the racemate [6, 12]. Blood concentration-time data were fitted to a three-compartment model with first-order oral absorption in order to get a robust estimate of λ_z and accurate non-compartmental pharmacokinetic parameters. The prolonged terminal

elimination half-life of *rac*-HCQ appeared to be predominantly due to its extensive distribution throughout the body. Its volume of distribution, approximating 8000 l after a single oral dose and 5500 l after the same dose injected i.v. [6], was much greater than total body water, which is consistent with the lipophilic nature of the HCQ molecule. When Tett and coworkers compared intra- and extra-vascular dosing [12], they failed to detect a statistical significance between elimination half-lives (44 ± 12 days i.v. *vs* 50 ± 16 days orally). The elimination half-life we calculated following a single oral dose was somewhat lower (23 ± 6 days), related to a higher estimate of HCQ total oral clearance (193 ml min^{-1}). Both studies measured HCQ levels in whole blood to assess correctly systemic blood clearance.

As observed after multiple doses [5], following a single oral dose of *rac*-HCQ, the blood concentrations of the R(−) enantiomer were always higher than those of the S(+)-enantiomer. The higher R(−)-HCQ concentrations are unlikely to result from the conversion of the S(+) enantiomer. In the rabbit, where the R/S ratios of HCQ blood concentrations are similar to those found in humans, the oral administration of either the racemate or the pure enantiomers also led to higher R(−)-HCQ blood concentrations, and there was no interconversion between enantiomers [13].

MacLachlan *et al.* [7] have shown that the time to maximum concentrations and the absorption half-life, assessed by deconvolution techniques, were similar for both enantiomers. However, since they sampled only for 32 h after dosing, they could not reliably account for the influence of the later distribution and elimination phases, which is essential for the accurate determination of the absorption rate. With a less extensive sampling schedule in the first few hours after dosing, we were able to confirm higher C_{max} values for R(−)-HCQ whereas t_{max} values were the same for both enantiomers. Since the early blood concentrations of each enantiomer are unlikely to be influenced by their very slow elimination half-life, these data may suggest different absorption half-lives.

HCQ enantioselective distribution appears to be, at least in part, secondary to a stereoselective binding to plasma proteins. The protein binding of HCQ enantiomers has never been assessed *in vivo*, but, *in vitro* using human plasma, R(−) and S(+)-HCQ were 37 and 64% protein bound, respectively [14]. R(−)-HCQ, with a 63% unbound fraction, would therefore be expected to have a higher volume of distribution than S(+)-HCQ. However, considering only plasma protein binding would negate the involvement of any binding to blood cells. This could be critical since HCQ appears to be accumulating not only in erythrocytes, but also in some white blood cells [13, 15]. It would be even more clinically relevant if these cells participate, directly or indirectly, in the pharmacological effects of the drug.

Since the enantiomeric composition of unresolved HCQ blood levels could not be determined beyond the first week after dosing, the elimination half-life of each enantiomer had to be derived from urine data. For *rac*-HCQ, the value of the elimination half-life calculated by this method was not significantly different from the one estimated from the blood concentration *vs* time curves (492 *vs* 563 h or 21 *vs* 23 days, respectively).

The shorter half-life of S(+)-HCQ appears to be partly explained by its faster renal clearance, which was 2.5 times higher than that of its antipode. Although the CL_R was calculated over 168 h, it is a reasonably accurate predictor of overall CL_R, since after 168 h, 88 to 92% of the total renal excretion of each enantiomer was already completed. Only small amounts continued to be excreted beyond the first week and they did not substantially alter the value of the amount excreted over 168 h. Consequently, the reversal in the enantiomeric composition of HCQ urine levels observed after 336 h in favour of the R enantiomer had only limited influence on the overall urinary excretion of HCQ: the total urinary excretion of R(−)-HCQ remained significantly inferior to that of S(+)-HCQ. Since the first 336 h account for 96% of HCQ urine excretion, the stereoselectivity in HCQ renal elimination may be reasonably described using the excretion rate of each enantiomer over the first 2 weeks.

Any stereoselectivity in HCQ renal elimination may also be secondary to differences in the plasma binding of the isomers as it affects glomerular filtration and passive reabsorption. After a single dose, the blood renal clearances of the R(−) and S(+) enantiomers, approximately 30 and 77 ml min^{-1} [our study], are of the same order of magnitude as those calculated at steady-state, 19 and 41 ml min^{-1} [5]. When steady-state plasma concentrations were corrected for the % binding to plasma proteins, MacLachlan *et al.* [5] reported that S(+)-HCQ unbound renal clearance was still 2.4 times higher than that of R(−)-HCQ. However, this would not account for possible differences in blood cells binding. Results were much higher than the glomerular filtration rate, implying that a significant part of HCQ renal excretion might be dependent upon tubular secretion. Therefore, it is possible that differences may be secondary to stereoselectivity in the process of active secretion and even in renal drug metabolism. However, renal elimination plays a minor role in the disposition of HCQ. Since no metabolites were detected in whole blood and low amounts were measured in urine, hepatic metabolism followed by biliary excretion appears to be the major route of HCQ elimination following a single oral dose.

The shorter half-life of S(+)-HCQ can also be attributed to its increased metabolism since the S forms of the metabolites were clearly predominant in urine. Oxidative *N*-dealkylation reactions are usually cytochrome P-450-dependent [16], but the enzymes responsible for HCQ metabolism remain unknown. Furthermore, it is not known if several isoforms are involved in the formation of a single metabolite or if each isoenzyme exhibits different stereochemical preferences.

The reversal in the enantiomeric composition of the urine reflects the blood concentrations, but also affects the enantiomeric composition of the meta-

bolite levels. Since S(+)-HCQ had a shorter half-life than R(−)-HCQ, its blood levels declined more rapidly, and as the amount in the body fell, so did its excretion rate and its metabolism. The excretion rate of S(+)-HCQ was eventually surpassed by that of R(−)-HCQ whereas S(+)-DCQ urine concentrations were gradually decreasing. Conversely, with DHCQ, a decreased elimination of the S(+) enantiomer was not observed, suggesting that DCQ and DHCQ production might be controlled by different enzymes. If the same enzyme was responsible for the production of both metabolites, one would expect similar trends in their urine excretion, provided that both have similar urine excretion rates (which is true from unresolved urine levels). Stereoselectivity in HCQ metabolism may therefore reflect the net balance of the affinity of different enzymes.

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