Effect of Experimental Diabetes Mellitus and Arthritis on the Pharmacokinetics of Hydroxychloroquine Enantiomers in Rats[1]

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Purpose. To study the effect of experimental diabetes and arthritis on the pharmacokinetics of hydroxychloroquine (HCQ) enantiomers in rats.

Methods. The pharmacokinetic studies were carried out following administration of 40 mg/kg of racemic HCQ to diabetic, insulin-treated diabetic, adjuvant arthritic and control rats.

Results. Renal (70% and 62% for R- and S-HCQ, respectively) and non-renal clearance (100% and 145% for R- and S-HCQ, respectively) of HCQ enantiomers were significantly increased in diabetic rats. Diabetes-induced alterations in the disposition of HCQ were reversed by insulin treatment. In arthritic rats, systemic clearance (CL) of HCQ enantiomers was significantly reduced (1.05 ± 0.15 and 1.3 ± 0.19 l/h/kg for R- and S-HCQ, respectively) compared to controls (1.69 ± 0.32 and 1.93 ± 0.34 l/h/kg for R- and S-HCQ, respectively). The fraction unbound of the R- and S-HCQ were 49.4% and 50.5% lower in platelet rich plasma of arthritic rats compared to healthy rats. Increased blood concentrations of HCQ enantiomers in arthritic rats were significantly related to the degree of inflammation.

Conclusions. Diabetes significantly increased the CL of both R- and S-HCQ by increasing renal and non-renal clearance. Arthritis caused a significant decrease in CL of HCQ enantiomers through increased binding and a decreased intrinsic clearance. The effect of the diseases on the pharmacokinetics of HCQ, however, was not stereoselective.

KEY WORDS: disposition; hydroxychloroquine enantiomers; experimental diabetes; arthritis.

INTRODUCTION

Hydroxychloroquine (HCQ) is a racemic antimalarial agent which is also an effective disease modifying drug against rheumatoid arthritis (1). A dramatic improvement in glucose and glycated hemoglobin has also been demonstrated in patients with non-insulin dependent diabetes mellitus (NIDDM) (2). Furthermore, we have recently demonstrated that HCQ reduces metabolic clearance of insulin and blood glucose levels by an HCQ concentration dependent manner in diabetic rats (3). The incidence of diabetes mellitus is greater in arthritic patients than in non-arthritic subjects (4). Thus, the co-existence of diabetes mellitus and rheumatoid arthritis may increase the risk of hypoglycemia where HCQ may sustain higher insulin levels in diabetic patients with some residual β-cell function.

Diabetes mellitus has been reported to induce an early increase in glomerular filtration in human (5) and animal models of diabetes (6,7), to alter the activity of metabolizing enzymes in humans (8) and animals (9,10) and to affect the blood concentrations of lipoproteins, proteins and free fatty acids (11,12). These pathophysiological consequences may potentially alter the pharmacokinetics and pharmacodynamics of HCQ.

Inflammatory conditions such as rheumatic disease are also associated with many pathophysiological changes which may alter drug disposition. Hypoalbuminemia (13) and increased acute phase reactant proteins such as α_1-acid glycoprotein (14), which are often observed in arthritic subjects, may affect protein binding of drugs. Acute inflammation also decreases hepatic drug metabolism (15,16). As the effect of HCQ on insulin and glucose metabolism is directly related to the HCQ blood concentration (3), alteration of pharmacokinetics of the drug in diseases for which the drug is indicated may have therapeutic consequences.

This study was designed to investigate the effect of streptozotocin-induced diabetes mellitus and adjuvant-induced arthritis on the pharmacokinetics of HCQ enantiomers in rats. The suitability of the rat as an animal model for the human was also evaluated.

EXPERIMENTAL SECTION

Materials

Streptozotocin (STZ), ortho-toluidine and chloroquine diphosphate (Sigma Chemical Co., St. Louis, MO); Insulin NPH (Iletin I, Eli Lilly and Company, Indianapolis, IN); racemic-HCQ sulfate (gift from Sepracor Inc., Marborough, MA); *Mycobacterium butyricum* (Difco Lab., Detroit, MI, USA). All other chemicals and solvents were either reagent or HPLC grade.

Animals

Adult male Sprague-Dawley rats were matched for their initial body weight and housed for at least 3 days before induction of the diseases. Animals had free access to standard laboratory rat chow and water. They were assigned to five groups as follows: diabetic (D) and control (C_D), insulin treated diabetic (ITD), adjuvant arthritic (AA) and control (C_A). A sixth group (PO) consisted of healthy rats that received the drug orally for determination of bioavailability.

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ABBREVIATIONS: AA, adjuvant arthritic, adjuvant arthritis; $AUC_{0-\infty}$, area under the concentration time curve from zero to infinity; AUC_{0-t}, area under the blood concentration time curve from zero to time t; AUMC, area under the first moment curve; C, control rats; CL, systemic clearance; Cl_{nr}, non-renal clearance; CL_r, renal clearance; C_{max}, maximum blood drug concentration; C_t, total drug plasma concentration (bound and unbound); C_{uf} ultrafiltrate drug concentration; D, diabetic rats; ΣXu, cumulative urinary excretion; F_u, fraction unbound; *g*, centrifugal force; GFR, glomerular filtration rate; HCQ, hydroxychloroquine; IDDM, insulin-dependent diabetes mellitus; ITD, insulin-treated diabetes; NIDDM, non-insulin dependent diabetes mellitus; STZ, streptozotocin; $t_{1/2}$, elimination half-life; T_{max}, time to reach maximum concentration; V_{ss}, volume of distribution at steady state.

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Induction of Diabetes Mellitus

Insulin-dependent diabetes mellitus (IDDM) was induced by a single i.p. injection of 60 mg/kg of STZ. To confirm the induction of disease, the serum concentration of glucose was measured on day four after injection of STZ and before administration of HCQ. Rats were considered diabetic and included in the study if their serum glucose was greater than 300 mg/dl. Commencing on the fourth day after induction of diabetes, each rat in the ITD group received a single daily injection of 15 IU/kg of insulin s.c. The last insulin injection was performed on day nine after the induction of diabetes (18).

Induction of Adjuvant Arthritis

To induce adjuvant arthritis, rats were innoculated intradermally into the tail base with 0.05 ml of heat-killed, freeze-dried *Mycobacterium butyricum* (Difco Lab, Detroit, USA) suspended in squalene (10 mg/ml). Rats were studied approximately 15 days later upon the appearance of arthritis. The degree of arthritis was assessed through measurement of hindpaw and forepaw swelling (16). An arthritis index score was obtained by grading each hindpaw on a 0 to 4 basis and each forepaw on a 0 to 3 basis. A maximum score of 14 could thus be obtained.

Surgery and Animal Maintenance

One day prior to HCQ administration, silastic catheters (0.58 mm i.d. × 0.965 mm o.d.; Clay Adams, Parsippany, NJ) were surgically implanted into the right jugular vein under light methoxyflurane anesthesia (Metofane, Pitman-Moore, Washington Crossing, NJ) and the animals were allowed to recover overnight. Rats had free access to water and were fasted for 16 h prior to drug administration.

Dosing and Sample Collection

Racemic HCQ dissolved in saline solution was administered i.v. (40 mg/kg base-equivalent) to C_D, C_A, D, ITD, and AA rats through the jugular vein catheter and p.o. to the PO group. We have previously shown that, in the rat, the insulin-sparing effect of HCQ commences at doses of 40 mg/kg/day (3). Blood samples (0.2 ml) were collected from the jugular vein cannula before and at 30 min, 1, 2, 3, 4, 6, 8, 12, 24, and 36 h after p.o. HCQ administration. Following the i.v. dose, two additional samples were also taken at 5 and 10 min post dose. All samples were stored at −20°C until analyzed. Total urine output was collected up to 108 h and, after recording the volume, an aliquot was stored and frozen for later analysis.

Binding to Plasma Protein and Platelets

The *in vitro* binding of HCQ enantiomers to protein and platelets was determined by an ultrafiltration technique using platelet-poor plasma and platelet-rich plasma from healthy and arthritic rats. For the collection of platelet-rich plasma the blood was anticoagulated with (Na_2) EDTA (Vacutainer tubes, Becton Dickinson, Rutherford, NJ) and centrifuged at 150 g for 10 min. The top layer, containing the platelet-rich plasma, was collected. Platelet-poor plasma was obtained after centrifugation of whole blood at 1800 *g* for 10 min. Three ml of platelet-poor plasma or platelet-rich plasma were spiked with racemic HCQ to yield concentrations of 0.5, 1, 2, 5, and 10 μg/ml. Ultrafiltration units (Diaflo membranes attached to Amicon ultrafiltration tubes, Micon Micropartition System, Amicon Div., W. R. Grace and Co. Danvers, MA) each containing 1 ml of sample were allowed to incubate at 37°C for 1 h. The units were then centrifuged at 1850 *g* for 45 min. The percent unbound of each HCQ enantiomer, F_u, was determined using the expression:

$$\%F_u = (C_{uf}/C_t)*100\%$$

where C_t was the total (bound and unbound) concentration of individual enantiomer in each sample before ultrafiltration, and C_{uf} was the ultrafiltrate HCQ concentration of each enantiomer.

Measurement of HCQ Enantiomers and Serum Glucose

Concentrations of HCQ enantiomers in blood and urine were measured using a previously described reversed-phase HPLC assay based on formation of diastereomers after derivatization with (+)-di-O-acetyl-L-tartaric anhydride and ultraviolet detection (19). The minimum quantifiable concentration of the assay was 10 ng/mL based on a 0.1 ml specimen. Serum glucose concentrations were determined using the ortho-toluidine method (20).

Pharmacokinetic Data Analysis

The data were best fitted to a biexponential equation using the computer program PCNONLIN 4.1 (SCI Software, Lexington, Kentucky). All estimates maintained a minimum correlation coefficient of 0.97. The area under the blood concentration-time curve ($AUC_{0-\infty}$ was calculated by the equation $A/\alpha + B/\beta$, where A and B were intercept coefficients, and α and β were the rate constants of the two disposition phases. The systematic clearance (CL) was calculated following i.v. doses from Dose/$AUC_{0-\infty}$, and the mean residence time (MRT) from $AUMC_{0-\infty}/AUC_{0-\infty}$, and the volume of distribution at steady state (V_{ss}) from CL* MRT. Oral bioavailability was calculated by dividing AUC after oral administration by AUC following i.v. dosing.

The urinary excretion rate of HCQ was calculated up to 108 h after dosing. The half-lives of HCQ enantiomers were estimated from the slope of the terminal linear portion of the log urinary excretion rate *vs* time (midpoint of the collection time) curve using the last three data points. Renal clearance (Cl_r) was determined as the slope of the cumulative urinary excretion of each HCQ enantiomer during the time interval from 0 to t *vs* the corresponding AUC_{0-t}, where t was the time at which the drug concentration was measured in whole blood. Non-renal clearance (CL_{nr}) was estimated for each rat by subtraction of CL_r from CL.

Statistical Analysis

Pharmacokinetic differences between AA and C_A rats and differences between pharmacokinetic parameters of individual enantiomers of HCQ were tested using unpaired and paired Student's two-sided t-test, respectively. The differences among the three groups of C_D, D, and ITD rats were determined based on a single factor analysis of variance (ANOVA). Further analysis of the means was achieved using Duncan's multiple range

test. All statistical tests were performed at 0.05 level of significance. Data are presented as mean ± S.D.

RESULTS

Induction of Diseases

Prior to induction of diseases, the body weights of the treated rats did not differ from their respective controls (Table I). However, 10 days after induction of diabetes, the body weights of D rats, but not AA rats were slightly but significantly lower compared to their respective controls. Serum glucose concentrations of diabetic rats were significantly higher than those of C_D and ITD rats just prior to the commencement of the pharmacokinetic experiment. Daily injection of 15 IU/kg of insulin, before commencement of the pharmacokinetic studies, resulted in normal serum glucose concentrations in the ITD animals. In the AA group, skin nodules on the ears and tail and swelling of the hind and fore paws were observed (arthritis index, 7.7 ± 4.1).

Pharmacokinetics of HCQ Enantiomers in Healthy Rats

The disposition of HCQ in blood was stereoselective, with the CL of S-HCQ being significantly higher than R-HCQ (Table II, Fig. 1). Half-lives of the enantiomers, calculated from the linear portion of the blood concentration-time course, were not different from each other (6.9 ± 1.3 and 7.5 ± 2.2 h for R- and S-HCQ, respectively). Half-lives estimated from urinary excretion rate *vs* time for both HCQ isomers, however, were longer than those calculated from blood data (30.2 ± 5.0 and 27.7 ± 5.6 h for R-and S-HCQ, respectively; P = 0.06, Fig. 1B). A significantly higher apparent volume of distribution was observed for S-HCQ as compared with R-HCQ after both i.v. and p.o. administration. The CL_r of S-HCQ was significantly higher than that of R-HCQ. There was also a trend towards higher CL_{nr} for S-HCQ as compared with the R enantiomer. Urinary excretion of the unchanged HCQ enantiomers was stereoselective (S>R).

Following oral dosing the difference in C_{max} between enantiomers was statistically significant (1.13 ± 0.37 and 0.94 ± 0.32 mg/l for R and S-HCQ, respectively), but there was no difference in the T_{max} values of the enantiomers (2.63 ± 1.1 and 2.00 ± 0.71 h, for R and S-HCQ, respectively; Fig. 1A). Similar to the i.v. doses, oral administration of HCQ resulted in a greater $AUC_{0-\infty}$ for R-HCQ (9.21 ± 1.33 $mg.l^{-1}.h$) as compared to S-HCQ (7.39 ± 1.35 $mg.l^{-1}.h$). The oral bioavailability of HCQ was not stereoselective (0.79 and 0.72 for R- and S-HCQ, respectively).

Effect of Diabetes Mellitus on Pharmacokinetics of HCQ Enantiomers

Diabetes resulted in a significant increase in CL of both enantiomers of HCQ (Fig. 1C, Table II), the effect being more pronounced for S-HCQ (100%) than for R-HCQ (80%). The increased CL and consequently decreased AUC, apparently resulted from a significant increase in both CL_r (70% and 63% for R- and S-HCQ, respectively) and CL_{nr} (100% and 145% for R- and S-HCQ, respectively). The decrease in V_{ss} of D rats (13% and 25% for R-HCQ and S-HCQ, respectively) was not significant compared to C_D rats. The diabetes-induced increase in CL was, therefore, responsible for the significant reduction in the half-lives of R- and S-HCQ (51% and 54% for R- and S-HCQ, respectively). Insulin treatment reversed diabetes-induced alterations in the pharmacokinetics of HCQ enantiomers (Table II). None of the pharmacokinetic parameters of HCQ enantiomers in ITD were statistically different from those in C_D rats.

Effect of Adjuvant Arthritis on the Pharmacokinetics of HCQ Enantiomers

The CL values for both enantiomers in AA rats were significantly lower than those in C_A rats (61% and 50% for R- and S-HCQ, respectively), resulting in significantly increased AUCs (Fig. 1D, Table II). Vss values of both enantiomers were significantly reduced by AA. R:S AUC ratios in AA rats did not differ significantly from C_A rats. Disease severity (arthritic index) correlated strongly with $AUC_{0-\infty}$ of both enantiomers (r = 0.90 and 0.83 for R and S-HCQ, respectively; Fig. 2).

Binding of HCQ enantiomers to platelets and plasma proteins was not concentration dependent within the examined concentration range (Fig. 3A). The mean unbound fraction (F_u) of HCQ in platelet poor plasma (Fig. 3B) showed stereoselectivity (0.62 ± 0.034 and 0.50 ± 0.065 for R- and S-HCQ, respectively). The order of this stereoselectivity was, however, reversed when platelet rich plasma was used (0.26 ± 0.015 and 0.32 ± 0.015 for R- and S-HCQ, respectively). The F_u values of the R-HCQ and S-HCQ were 49.4% and 50.5% lower in platelet rich plasma of arthritic rats as compared to that of normal rats (Fig. 3B). The correlation was observed between

Table I. Body Weights and Serum Glucose Levels in Diabetic (D), Insulin-Treated Diabetic (ITD), Adjuvant Arthritic (AA) Rats, and Their Respective Controls (C_D and C_A)

	Diabetic			Arthritic	
	C_D	D	ITD	C_A	AA
Body weight (g)					
Before induction of disease	260 ± 20	274 ± 31	258 ± 21	263 ± 25	258 ± 18
At the time of PK studies	343 ± 36	310 ± 33[a]	360 ± 15	403 ± 30	386 ± 28
Fasting serum glucose (mg/dl)	139 ± 5	414 ± 49[b]	116 ± 34	NA[c]	NA

[a] Significantly different from C_D.
[b] Significantly different from C_D and ITD.
[c] NA, Not applicable.

Table II. Pharmacokinetic Parameters of HCQ in Diabetic (D), Insulin-Treated Diabetic (ITD), Adjuvant Arthritic (AA) Rats, and Their Respective Controls (C_D and C_A)

Indices	Diabetic		Arthritic			Statistical analysis			
	C_D	D	ITD	C_A	AA	C_D vs D	C_D vs ITD	D vs ITD	$C_A$vsAA
				R-HCQ					
$AUC_{0-\infty}$ (mg.l^{-1}.h)	12.1 ± 2.31[a]	6.8 ± 1.47[a]	11.6 ± 1.47	12.8 ± 1.85[a]	19.5 ± 3.12[a]	+	−	+	+
CL_{TB} (l/h/kg)	1.69 ± 0.32[a]	3.04 ± 0.69[a]	1.74 ± 0.22	1.61 ± 0.25[a]	1.05 ± 0.15[a]	+	−	+	+
CL_r (l/h/kg)	0.61 ± 0.14[a]	1.04 ± 0.13	0.72 ± 0.17	ND	ND	+	−	+	ND
CL_{nr} (l/h/kg)	1.00 ± 0.15[a]	2.00 ± 0.57[a]	1.02 ± 0.16	ND	ND	+	−	+	ND
$Xu_{(0-108)}$ (% dose)	28.8 ± 0.74	26.7 ± 3.29	27.4 ± 1.52	ND	ND	−	−	−	ND
$\beta t_{1/2}$ (h)	6.95 ± 1.30	3.40 ± 1.24	5.64 ± 0.93	7.12 ± 1.23	7.91 ± 1.99	+	−	+	−
V_{as} (l/kg)	14.6 ± 2.74[a]	12.6 ± 2.47	13.0 ± 2.61	14.4 ± 3.34[a]	10.6 ± 2.09[a]	−	−	−	+
				S-HCQ					
$AUC_{0-\infty}$ (mg.l^{-1}.h)	10.6 ± 1.93[a]	10.5 ± 2.05[a]	5.42 ± 1.43[a]	10.2 ± 1.77	15.8 ± 2.18[a]	+	−	+	+
CL_{TB} (l/h/kg)	1.93 ± 0.34[a]	1.96 ± 0.33[a]	3.90 ± 1.08[a]	2.01 ± 0.33	1.30 ± 0.19[a]	+	−	+	+
CL_r (l/h/kg)	0.80 ± 0.21[a]	ND	1.30 ± 0.28	0.96 ± 0.24	ND	+	−	+	ND
CL_{mr} (l/h/kg)	1.06 ± 0.13[a]	ND	2.60 ± 0.85[a]	1.05 ± 0.16	ND	+	−	+	ND
$Xu_{(0-108)}$ (% dose)	34.3 ± 1.17	ND	31.0 ± 4.38	32.5 ± 2.38	ND	−	−	−	ND
$\beta t_{1/2}$ (h)	7.5 ± 2.2	7.01 ± 1.03	3.4 ± 1.24	5.40 ± 1.02	8.66 ± 2.80	+	−	+	−
V_{as} (l/h/kg)	17.2 ± 4.04[a]	16.5 ± 3.47[a]	12.8 ± 3.93	13.9 ± 3.17	13.6 ± 3.74[a]	−	−	−	+

[a] Significantly different from other enantiomer, (+) Significantly different; (−) Not significantly different; (ND) Not determined.

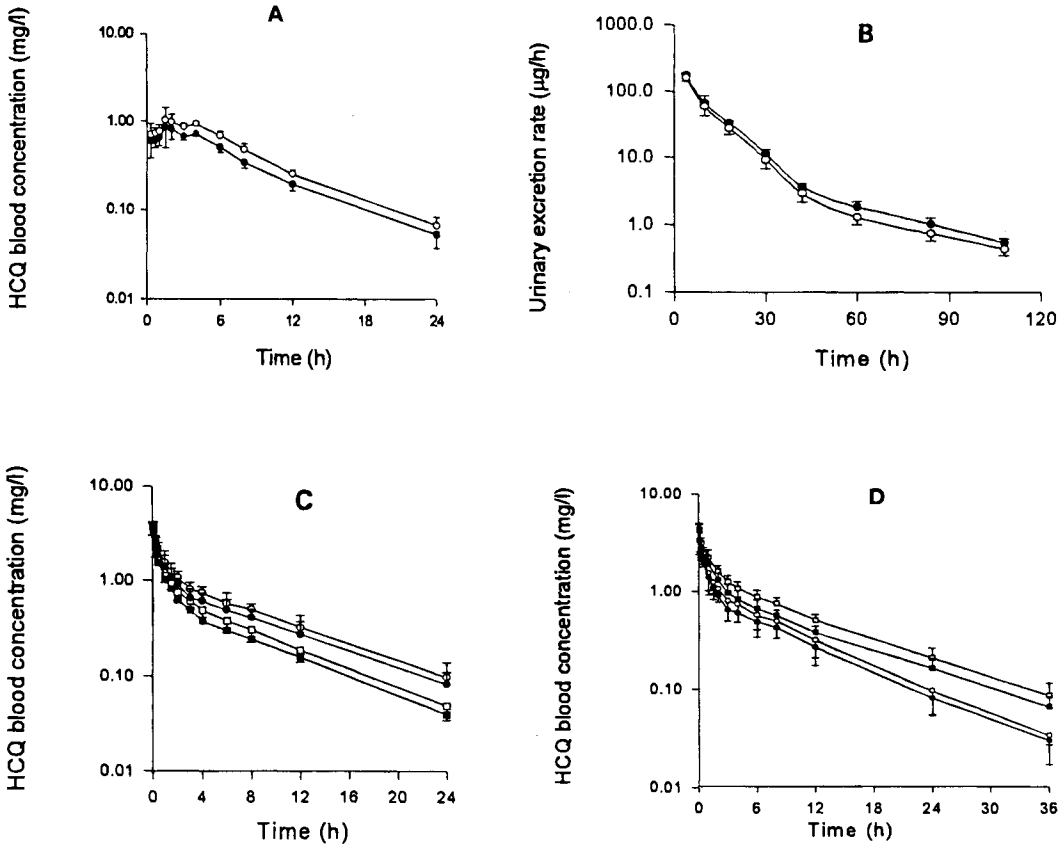

Fig. 1. (**A**) Mean (±SD) blood concentration-time profiles of R-HCQ (○) and S-HCQ (●) following oral administration to control rats; (**B**) Mean urinary excretion rate-time curves of R-HCQ (○) and S-HCQ (●) following i.v. administration to control rats; (**C**) Blood concentration-time profiles of HCQ enantiomers following i.v. administration to control (R-HCQ, ○; S-HCQ, ●) and diabetic (R-HCQ; □, S-HCQ, ■). (**D**) Blood concentration-time profiles of HCQ enantiomers control (R-HCQ, ○; S-HCQ, ●) and arthritics (R-HCQ, □; S-HCQ, ■) rats. All doses, single 40 mg/kg racemic HCQ, n = 5-6.

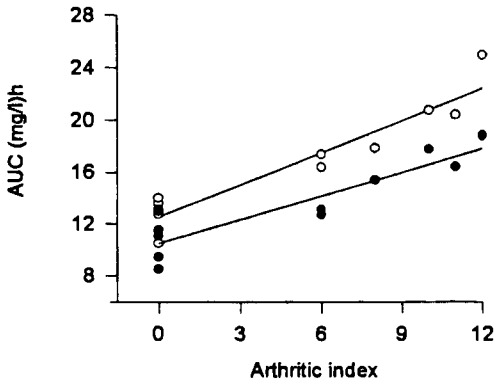

Fig. 2. Area under the blood concentration-time curves R-HCQ (○) and S-HCQ (●). HCQ *vs* arthritic index.

the number of platelets in plasma and F_u for both R (r = 0.85) and S (r = 0.81) enantiomers of HCQ (Fig. 3C).

DISCUSSION

Blood and urinary excretion data suggested a two and three open compartment model for pharmacokinetics of HCQ enantiomers in rats, respectively. The discrepancy is attributed to the fact that the time-course of the drug in blood could not be followed for a sufficient length of time due to limitations in assay sensitivity. Urinary excretion data, therefore, revealed longer half-lives for both HCQ isomers (30.2 ± 5.0 and 27.7 ± 5.6 h for R-and S-HCQ, respectively) compared to those obtained from blood concentration-time courses (6.9 ± 1.3 and 7.5 ± 2.2 for R- and S-HCQ, respectively) (Fig. 1). It is, therefore, reasonable to suggest that disposition of HCQ enantiomers in the rat follows a three compartment model as has been reported in humans (17,21). Nevertheless, the extrapolated ($AUC_{t-\infty}$ estimated using the longer t1/2 from the urinary data was only less that 10% of the total AUC. The volume of distribution of HCQ enantiomers was found to be 25 times greater than rat total body water. This is qualitatively parallel to that observed in human studies (17,21). As observed after single doses of rac-HCQ to humans (17), higher blood concentrations of R-HCQ compared with S-HCQ were seen in the rat model. The mean R:S AUC ratio after p.o. administration of the drug observed in rats was close to that observed in humans (1.3 *vs* 1.8 respectively). As previously reported with humans (22), our *in vitro* rat protein binding studies indicate that the binding of HCQ to rat plasma protein is enantioselective in favor of S-HCQ (Fig. 3). In light of this similarity with humans, the rat appears to be a good model for further pharmacokinetic studies of HCQ enantiomers.

The pathophysiological changes and alterations in glucose homeostasis associated with diabetes mellitus may influence basic cellular processes thereby altering the pharmacokinetics and pharmacodynamics of drugs. An elevated glomerular filtration rate (GFR) in the early phase of insulin-dependent diabetes mellitus has been reported in diabetic humans (5) and animal models of diabetes (6,7). The elevation of GFR persists during the first decade of disease despite insulin therapy and adequate metabolic control. Subsequently, GFR significantly decreases in patients with long-standing diabetes (5). The observed STZ-induced diabetes resulted in a significant increase in renal clearance of HCQ enantiomers through an accelerated GFR as has been reported for STZ-induced diabetic rats (6).

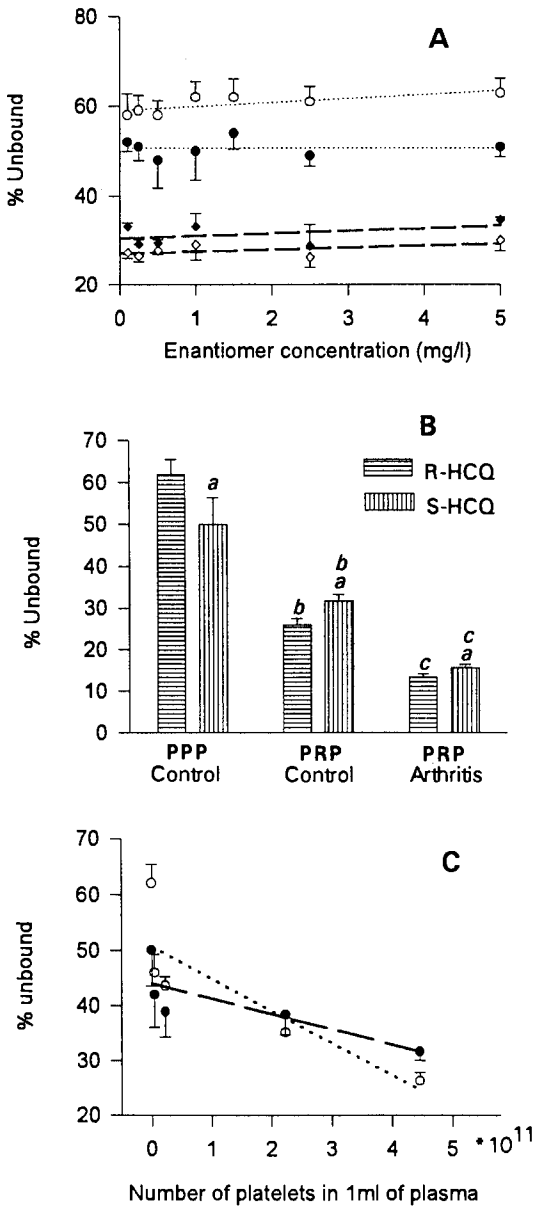

Fig. 3. (**A**) Mean (±SD) percent unbound *vs* total drug concentration in platelet-poor plasma (R-HCQ, ○···○; S-HCQ, ●···●) and platelet-rich plasma (R-HCQ, ○---○; S-HCQ, ●---●). (**B**) Mean (±SD) percent unbound of HCQ enantiomers in platelet-poor plasma (PPP) and platelet-rich plasma (PRP) of control; arthritic rats. (**C**) Mean (±SD) percent unbound of R-HCQ (○) and S-HCQ (●) as a function of platelet numbers in rat plasma. *a*, Significantly different from R-HCQ; *b*, Significantly different from platelet-poor plasma of controls; *c*, Significantly different from platelet-rich plasma of controls; n = 3 in all experiments.

Hepatic drug metabolism, mediated by cytochrome P-450 enzymes, is altered in certain disease states, including diabetes mellitus, in both humans (8) and diabetic animals (9,10,23). The direction and extent of the diabetic alteration in metabolism, however, are dependent on several factors such as acuteness and type of the disease, duration of diabetes, gender, and the

substrate under study. For instance, elimination of antipyrine, a marker for hepatic intrinsic clearance, was found to be faster in patients with treated IDDM (8) and in STZ-induced diabetic rats (9). Metabolism of aminopyrine, on the other hand, was shown to be slower in both alloxan- and STZ-induced diabetic male rats (23) but faster in female rats (10). Our results indicate that STZ-diabetes causes a significant increase in CL_{nr} of both HCQ enantiomers (Table II). The total drug excreted in urine indicates that about 70% of the administered dose is eliminated through non-renal pathways in diabetic and non-diabetic rats, presumably via hepatic metabolism. The observed increased CL of HCQ, therefore, is likely due to enhanced efficiency of both renal and non-renal clearances of the drug caused by diabetes.

Blood concentrations of lipoproteins and proteins are known to be different in diabetic and control rats (12). It is also well known that free fatty acids are elevated in the plasma of patients with either IDDM or NIDDM (11). Elevated free fatty acids, and glycosylation of plasma protein in diabetes mellitus may affect the extent of binding of basic and acidic drugs. In addition, extra- and intracellular volumes are elevated in experimental diabetes mellitus (7). Hence, the volume of distribution of drug may be altered in diabetic states. We, however, did not observe a significant difference in the volume of distribution between diabetic and control rats (Table II).

Inflammatory conditions are associated with decreased plasma albumin and increased α_1-acid glycoprotein concentrations (13). Since basic drugs have an affinity for α_1-acid glycoprotein, their binding is often increased in diseases such as arthritis (14). This may result in reduced clearance. The impairment of drug metabolism in humans with inflammatory diseases (24) and experimental models of inflammation has been well established (16,25). Liver microsomal oxidative enzyme activity and cytochrome P-450 content are reduced following induction of adjuvant arthritis in rats (15). Propranolol levels have been reported to be significantly higher than normal in rats afflicted with inflammation (25). These increases have been explained by both decreased metabolism and/or increased protein binding. For highly extracted drugs such as propranolol, a reduced hepatic blood flow may also result in reduced CL. The low hepatic extraction ratio of HCQ enantiomers excludes the likelihood of a mechanism involving hepatic blood flow. Furthermore, it has been shown that hepatic blood flow remains unchanged in inflammation (28).

Hydroxychloroquine, a weak base which moderately binds to plasma proteins, has a low hepatic extraction ratio in humans (21) and rats (Table II). We have previously reported a significant inverse correlation between unbound fraction of HCQ in blood and blood α_1-acid glycoprotein concentration in arthritic humans (26). The drug is also extensively bound to platelets in both species (26, Fig. 3B and 3C). It has been shown that the number of platelets rises in active rheumatoid arthritis proportional to both clinical and laboratory parameters (27). In both man and rats, HCQ elimination is mainly dependent on biotransformation in the liver (21). Hence, the elevation in both α_1-acid glycoprotein and platelets and subsequent decrease in Vss, coupled with reduction in the hepatic intrinsic ability to metabolize the drug, are likely explanations for the observed higher HCQ blood concentrations.

The progressively elevated AUCs of HCQ enantiomers with increased inflammation (Fig. 2) indicate that a strong relationship exists between disease severity and reduced HCQ clearance. Such a relationship has also been demonstrated for propranolol (29). It is likely that a similar relationship between the magnitude of inflammation and altered HCQ disposition exists in humans. Therefore, patients with severe arthritis, who are taking HCQ, could have higher than normal blood concentrations. The therapeutic significance of these finding remains to be explored.

It is also worth mentioning that for drugs that are bound to platelets, attention must be paid to the number of platelets in the specimen. This number is readily influenced by the force and/or length of centrifugation (Fig. 3B and 3C). For example, analysis of plasma and serum may yield entirely different results due to the difference in their platelet counts (26).

In conclusion, our study showed that the rat is an appropriate animal model for delineating the pharmacokinetics of HCQ enantiomers. Experimentally induced diabetes significantly increased the CL of both enantiomers of HCQ by increasing renal and non-renal clearance. Adjuvant-induced arthritis, on the other hand, caused a significant decrease in CL of HCQ enantiomers through increased binding and decreased intrinsic clearance. The effect of the diseases, however, was not stereoselective as both enantiomers were affected almost equally. Increased blood concentrations of HCQ enantiomers in arthritic rats were found to be significantly related to the degree of inflammation.

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REFERENCES

1. The HERA study group. A randomized trial of hydroxychloroquine in early rheumatoid arthritis. *Am. J. Med.* **98**:156–168 (1995).
2. A. G. Quatraro, M. Consoli, F. Magno, F. Carreta, F. Nardoza, A. Ceriello, and D. Giugliano. Hydroxychloroquine in decompensated, treatment-refractory noninsulin-dependent diabetes mellitus. *Ann. Intern. Med.* **112**:678–681 (1990).
3. J. Emami, F. M. Pasutto, and F. Jamali. Insulin-sparing effect of hydroxychloroquine in experimental diabetes mellitus. *Pharm. Res.* Suppl. **12**(9):S-409 (1995).
4. S, Martin, J. Kardorf, B. Schulte, E. F. Lampeter, F. A. Gries, I. Malchers, R. Wagner, J. Bertrams, B. O. Roep, and A. Pfutzner. Autoantibodies to the islet antigen ICA69 occur in IDDM and in rheumatoid arthritis. *Diabetologia* **38**:351–355 (1995).
5. C. E. Mogensen. Glomerular filtration rate and renal plasma flow in short-term and long-term juvenile diabetes mellitus. *Scand. J. Clin. Lab. Invest.* **28**:91–100 (1971).
6. K. Jensen, J. S. Christiansen, A. K. Asteven, and H. H. Parving. Renal function in streptozotocin-diabetic rats. *Diabetologia* **21**:409–414 (1981).
7. J. N. Harvey, A. A. Jaffa, C. B. Loadhalt, and R. K. Mayfield. Measurement of glomerular filtration rate and renal plasma flow in the diabetic rat by the single-injection isotopic technique: effects of altered distribution volumes of ^{51}Cr-EDTA and ^{125}I-hippuran. *Diabetes Res.* **9**:67–72 (1988).
8. T. Zysset and H. Wietholtz. Differential effect of type I and type II diabetes on antipyrine disposition in man. *Eur. J. Clin. Pharmacol.* **34**:369–375 (1988).
9. V. Mahachai, S. Hutchison, M. Keelan, and A. B. R. Thomson. *In vivo* hepatic microsomal function in diabetic rats. *Clin. Pharmacol. Ther.* **43**:174 (1988).
10. R. Kato, K. Onoda, and A. Takanaka. Species difference in drug metabolism by liver microsomes in alloxan diabetic or fasted animals (I) the activity of drug-metabolizing enzymes and electrone transpoert systems. *Jap. J. Pharmacol.* **20**:546–553 (1970).
11. E. Fraze, C. C. Donner, A. L. M. Swislocki, Y-A. M. Chiou,

Y-D. I. Chen, and G. M. Reaven. Ambient plasma free fatty acid concentrations in non-insulin-dependent diabetes mellitus: Evidence for insulin resistance. *J. Clin. Endocrin. Metab.* **61**:807–811 (1985).
12. D. R. Miller, D. E. Treat, B. Fridd, and D. Wemett. Effect of streptozotocin diabetes in the rat on blood levels of ten specific plasma proteins and on their net biosynthesis by the isolated perfused liver. *Hepatology* **11**:635–645 (1990).
13. F. A. Van Den Ouweland, M. J. Franssen, L. B. Van De Putte, Y. Tan, C. A. Van Ginneken, and F. W. Gribnau. Naproxen pharmacokinetics in patients with rheumatoid arthritis during active polyarticular inflammation. *Br. J. Clin. Pharmacol.* **23**:189–193 (1987).
14. F. M. Belpaire, M. G. Bogaert, and M. Rosseneu. Binding of β-adrenoreceptor blocking drugs to human serum albumin, to α_1-acid glycoprotein and to human serum. *Eur. J.Clin. Pharmacol.* **23**:246–253 (1982).
15. M. Ishikawa, K. Sasaki, M. Ozaki, K. Watanabe, and Y. Takayangi. Hepatic drug metabolizing activity in rats with carageenan-induced inflammation. *J. Pharmacobio. Dyn.* **14**:132–138 (1991).
16. M. Piquette-Miller and F. Jamali. Effect of adjuvant arthritis on the disposition of acebutalol enantiomers in rats. *Agents and Actions.* **37**:290–296 (1992).
17. J. Ducharme, H. Fieger, M. P. Ducharme, S. K. W. Khalil, and I. W. Wainer. Enantioselective disposition of hydroxychloroquine after a single oral dose of the racemate to healthy subjects. *Br. J. Pharmacol.* **40**:127–133 (1995).
18. R. Mehvar. Effect of experimental diabetes mellitus on the pharmacokinetics of atenolol enantiomers in rats. *J. Pharm. Sci.* **80**(3):207–211 (1991).
19. D. R. Brocks, F. M. Pasutto, and F. Jamali. Analytical and semi-preparative high-performance liquid chromatographic separation and assay of hydroxychloroquine enantiomers. *J. Chromatogr.* **581**:83–92 (1992).
20. J. J. Linne and K. M. Ringsrud. o-Toluidine glucose determination. In *Basic Techniques for the Medical Laboratory.* Second Edition. pp. 129–131, McGraw-Hill Book Company, New York (1979).
21. S. E. Tett, D. J. Cutler, and R. O. Day. A dose-ranging study of the pharmacokinetics of hydroxychloroquine following i.v. administration to healthy volunteers. *Br. J. Clin. Pharmacol.* **26**:303–313 (1988).
22. A. J. McLachlan, D. J. Cutler, and S. E. Tett. Plasma protein binding of the enantiomers of hydroxychloroquine and metabolites. *Eur. J. Clin. Pharmacol.* **44**:481–484 (1993).
23. A. Toda, H. Shimeno, and A. Nagamatsu. Effect of experimental diabetes on aminopyrine metabolism in rats. *Xenobiotica* **17**:1075–1083 (1987).
24. R. E. Schneider and H. Bishop. β-blocker plasma concentrations and inflammatory disease: clinical implications. *Clin. Pharmacokinet* **7**:281–284 (1982).
25. M. Piquette-Miller and F. Jamali. Selective effect of adjuvant arthritis on the disposition of propranolol enantiomers in rats detected using a stereospecific HPLC assay. *Pharm. Res.* **10**(2):294–299 (1993).
26. D. R. Brocks, K. J. Skeith, C. Johnston, J. Emami, P. Davis, A. S. Russell, and F. Jamali. Hematologic disposition of hydroxychloroquine enantiomers. *J. Clin. Pharmacol.* **34**:1088–1097 (1994).
27. M. Farr, D. L. Scott, T. J. Constable, R. J. Hawker, C. F. Hawkins, and J. Stuart. Thrombocytosis of active rheumatoid disease. *Annal Rheum. Disease* **42**:545–549 (1983).
28. K. A. Walker, H. E. Barber, and G. M. Hawkworth. Mechanism responsible for altered propranolol disposition in adjuvant-induced arthritis in the rat. *Drug. Metab. Disposit.* **14**:482–486 (1986).
29. M. Piquette-Miller and F. Jamali. Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofen-treated and untreated adjuvant arthritis. *Drug Metab. Disposit.* **23**(2):353–358 (1995).