Eur J Clin Pharmacol (1993) 44: 481–484

European Journal of Clinical Pharmacology

Plasma protein binding of the enantiomers of hydroxychloroquine and metabolites

A. J. McLachlan[1,*], D. J. Cutler[1], and S. E. Tett[2]

[1] Department of Pharmacy, University of Sydney, NSW, Australia
[2] Department of Clinical Pharmacology and Toxicology, St Vincent's Hospital, Darlinghurst, NSW, Australia

Received: June 19, 1992/Accepted in revised form: January 8, 1993

Summary. The *in vitro* binding of the enantiomers of hydroxychloroquine and its three major metabolites in pooled plasma obtained from four healthy volunteers and the binding of the enantiomers of hydroxychloroquine to purified plasma proteins has been investigated.

The plasma protein binding of hydroxychloroquine was found to be stereoselective. The (S)-enantiomer of hydroxychloroquine was 64% bound in plasma, while (R)-hydroxychloroquine was 37% bound. Fifty% of (S)-hydroxychloroquine was bound to a 40 $g \cdot l^{-1}$ solution of human serum albumin, while only 29% of the (R)-enantiomer was bound. The enantioselectivity of hydroxychloroquine binding was reversed in a 0.7 $g \cdot l^{-1}$ solution of α_1-acid glycoprotein with (R)-hydroxychloroquine being bound to a greater extent than its optical antipode (41% versus 29%). The enantiomers of the metabolites of hydroxychloroquine were bound to a similar extent to plasma and purified plasma proteins.

Binding of hydroxychloroquine to plasma and purified proteins was found to be linear over the racemic concentration range of 50 to 1000 $ng \cdot ml^{-1}$ and hydroxychloroquine metabolite binding to plasma was linear over the range 25 to 500 $ng \cdot ml^{-1}$.

Key words: Hydroxychloroquine; enantiomers, protein binding, metabolites

Hydroxychloroquine is used as a racemate in the management of rheumatoid arthritis. There is no data in the literature regarding the relative pharmacodynamic activity of hydroxychloroquine enantiomers in rheumatoid disease. Preliminary results from our laboratory indicate that hydroxychloroquine disposition is stereoselective [McLachlan et al. 1991]. The protein binding of rac-hydroxychloroquine has been investigated [Tett et al. 1988], but the protein binding of the metabolites and the enantiomers of hydroxychloroquine have not been previously reported.

This paper reports the findings of a study of the *in vitro* protein binding of the enantiomers of hydroxychloroquine and metabolites in pooled plasma from healthy subjects. The protein binding of (R)- and (S)-hydroxychloroquine have also been investigated in the presence of human serum albumin and α_1-acid glycoprotein.

Materials and methods

Heparinised drug-free blood from four healthy subjects was centrifuged at 1200 g for 10 min and the pooled plasma was stored frozen at −18 °C. Krebs solution (pH 7.4) was prepared using 6.90 g NaCl, 0.35 g KCl, 0.29 g $MgSO_4 \cdot 7H_2O$, 0.16 g KH_2PO_4, 2.10 g $NaHCO_3$ (all from Ajax Chemicals, Sydney, Australia), 0.37 g $CaCl_2 \cdot 2H_2O$ (BDH Chemicals, Port Fairy, Australia) and 2.00 g (D(+)-glucose (May & Baker, Footscray, Australia) in 1000 ml of freshly distilled water.

Protein solutions were prepared using human serum albumin (Fraction V) and α_1-acid glycoprotein (both from Sigma Chemical Co., St Louis, USA) in Krebs solution.

Racemic hydroxychloroquine sulphate, desethylhydroxychloroquine and desethylchloroquine were supplied by Sterling Pharmaceuticals Pty Ltd (Sydney, Australia) bisdesethylchloroquine was provided by the Army Malaria Research Unit (Ingleburn, Australia), and chloroquine diphosphate (used as the internal standard for the HPLC assay) was obtained from Sigma Chemical Company (St. Louis, MO, USA).

Chloroquine has been reported to bind to some plastics [Yahya et al. 1986] and glass surfaces [Geary et al. 1983]. All glassware coming in contact with hydroxychloroquine and its metabolites was treated with Aquasil silanising liquid (Pierce, Rockford, IL, USA) to prevent adsorption.

Protein binding was assessed in pooled drug-free plasma from healthy subjects, 40 g/l human serum albumin in Krebs solution, 0.7 g/l α_1-acid glycoprotein in Krebs solution.

The protein binding of the enantiomers of hydroxychloroquine was determined at the following concentrations of rac-hydroxychloroquine; 50, 100, 250, 500 and 1000 $ng \cdot ml^{-1}$ in pooled plasma; 50, 100, 500 and 1000 $ng \cdot ml^{-1}$ in a solution of human serum albumin; 50, 250, 500 and 1000 $ng \cdot ml^{-1}$ in a solution of α_1-acid glycoprotein.

The protein binding of the enantiomers of hydroxychloroquine metabolites (desethylhydroxychloroquine, desethylchloroquine, bisdesethylchloroquine) were assessed at the racemic concentra-

* *Present address:* Department of Pharmacy, University of Manchester, Manchester, UK

tions of 25, 50, 100 and 500 ng·ml^{-1} in pooled plasma. The protein binding of the racemic metabolites was determined at 25, 50 and 100 ng·ml^{-1} in a solution of human serum albumin and at 500 ng·ml^{-1} in a solution of α_1-acid glycoprotein.

Perspex dialysis cells (2.0 ml) were used to separate free and bound drug. Protein solutions or plasma were dialysed against Krebs solution (pH 7.4) at 37°C. Dialysis temperature was maintained by emersing the dialysis cells in a temperature controlled water bath (Thermomix, Braun, model 1441). Visking Cellulose type 20 (Union Carbide) dialysis membrane was used to separate protein and Krebs solution in the perspex cells. Dialysis membranes were prepared by boiling for 5 minutes in two portions of distilled water, and were left soaking in distilled water until the time of dialysis.

Plasma and protein samples were dialysed for 3 h and 6 h to establish that equilibrium was reached. Racemic hydroxychloroquine or metabolite was added to both sides of the dialysis cell with 60% of drug on the protein side and 40% on the Krebs solution side (which approximates the ratio expected at equilibrium).

The volume of each half-cell was measured after dialysis to check for any volume shifts within the cell. Samples were discarded if a volume shift, dialysis membrane damage or protein leakage were apparent. The protein binding was determined in triplicate for all compounds at each concentration.

At equilibrium both sides of the dialysis cells were assayed in duplicate for racemic compound and enantiomers. The assay of McLachlan et al. (1991) for the enantiomers of hydroxychloroquine and metabolites was used in the present study.

Data analysis

The percentage of drug bound for racemic drug and enantiomers was determined using the following equation,

$$\%\ bound = \frac{C_{protein} - C_{buffer}}{C_{protein}} \times 100$$

where $C_{protein}$ is the concentration of racemate or enantiomer on the protein or plasma side (bound + free drug) and C_{buffer} is the concentration on the Krebs solution side (free drug).

Statistical comparisons were made using a paired Student's *t*-test (Epistat statistical programme).

Results

The estimated free fractions at both dialysis times (3 and 6 h) were not different ($P = 0.12$), indicating that equilibration was reached by 3 h. Using similar equilibrium dialysis apparatus, Walker et al. (1983) found that the protein binding of rac-chloroquine reached equilibrium after 2 h.

Table 1 shows a summary of the *in vitro* protein binding of the racemate and enantiomers of hydroxychloroquine (HCQ), desethylhydroxychloroquine (HCQM), desethylchloroquine (CQM) and bisdesethylchloroquine (CQMM) in pooled plasma of 4 healthy subjects and in purified plasma protein solutions. Linearity was also established for the purified plasma protein binding of the racemate of metabolites over the concentration range 25 ng·ml^{-1} to 500 ng·ml^{-1} in plasma and protein solutions.

In the pooled plasma of healthy subjects the percentage of the (S)-enantiomer bound was greater than that of its optical antipode for all compounds. The mean ratio ((S)-/(R)-) of the protein binding of the enantiomers of hydroxychloroquine in pooled plasma from healthy subjects was 1.73. The same pattern of stereoselectivity, with (S)-hydroxychloroquine being bound to a greater extent, was observed for the protein binding of hydroxychloroquine enantiomers in a solution of 40 g·l^{-1} human serum albumin. The ratio ((S)-/(R)-) of the protein binding of the enantiomers of hydroxychloroquine in a solution of human serum albumin was 1.77. The pattern of protein binding in a solution of 0.7 g·l^{-1} α_1-acid glycoprotein was the reverse of that observed for the protein binding to plasma and a solution of human serum albumin; the ratio ((R)-/(S)-) of the protein binding of the enantiomers of hydroxychloroquine to α_1-acid glycoprotein was 1.43.

Table 1. The *in vitro* protein binding of the racemate and enantiomers of hydroxychloroquine (HCQ), desethylhydroxychloroquine (HCQM), desethylchloroquine (CQM) and bisdesethylchloroquine (CQMM) in plasma purified protein solutions

Compound	n[a]	Percentage bound in vitro (mean ± SD) Racemic	R	S	t-test[b]
Pooled healthy plasma					
HCQ	9	52 (7)	37 (8)	64 (8)	$P < 0.0001$
HCQM	10	49 (9)			
	5		37 (12)	56 (9)	$P = 0.008$
CQM	10	55 (4)			
	5		42 (6)	65 (5)	$P = 0.009$
CQMM	8	52 (6)			
	5		30 (10)	60 (10)	$P = 0.014$
40 g/l human serum albumin solution					
HCQ	10	40 (5)			
	9		29 (6)	50 (5)	$P < 0.0001$
HCQM	7	45 (5)			
CQM	4	23 (5)			
CQMM	4	28 (9)			
0.7 g/l α_1-acid glycoprotein solution					
HCQ	9	34 (6)	41 (6)	29 (8)	$P < 0.0001$
HCQM	3	33 (5)			
CQM	3	34 (3)			

[a] The number of determinations
[b] P value determined using a Student's paired t-test comparing mean binding of (R)- versus (S)-enantiomers
[c] Empty cells in table not determined

Due to the limited quantities of metabolites available the protein binding for the enantiomers of the hydroxychloroquine metabolites was not determined in the protein solutions. The protein binding of rac-bisdesthylchloroquine was not determined in α_1-acid glycoprotein solutions.

Discussion and conclusions

The concentration ranges used in the present study reflect the range of racemic plasma concentrations for hydroxychloroquine and metabolites observed following a single dose [Tett et al. 1988; Miller et al. 1991] and during multiple dosing [Tett et al. 1990a]. The concentrations of human serum albumin (40 g/l) and α_1-acid glycoprotein (0.7 g·l^{-1}) used in this study were chosen to reflect the concentrations of these proteins observed in the plasma of healthy individuals [Piafsky et al. 1978].

The present findings for the binding of rac-hydroxychloroquine to plasma are in agreement with the data of Tett et al. (1988) who found that 45 (3) % of rac-hydroxychloroquine was bound in plasma obtained from one healthy volunteer and that this value was constant over the concentration range 50 ng · ml^{-1} to 1000 ng · ml^{-1}. In the present study it was found that protein binding was also constant over the range 50 ng · ml^{-1} to 1000 ng · ml^{-1} in 4 % human serum albumin (mean (SD) 40 (5) %).

The enantioselectivity in binding in plasma was the same as in a solution of albumin, the opposite of that for α_1-acid glycoprotein, suggesting that, at normal levels of α_1-acid glycoprotein, the overall protein binding of the enantiomers of hydroxychloroquine in plasma is largely determined by binding to albumin.

The binding of the enantiomers of the parent drug and the metabolites were studied in the presence of the optical antipode. It is possible that *in vivo* competition for binding sites between the parent drug, metabolites and their enantiomers could result in altered binding from that observed in the present *in vitro* studies. The linearity of the relationship between percentage bound and concentration of enantiomer suggests that the concentrations used in the present study (and observed clinically) are well below the concentration required to saturate hydroxychloroquine protein binding sites. It is therefore not expected that competition effects would be significant.

The findings of this study show the same binding pattern as for the enantiomers of chloroquine [Ofori-Adjei et al. 1986]. The ratio of the protein binding for the enantiomers of chloroquine in pooled plasma and solutions of purified plasma proteins were not different from that observed for hydroxychloroquine in the present study. Ofori-Adjei et al. (1986) investigated the protein binding of rac-desethylchloroquine in plasma and the observed value of 57 (3) % bound was similar to the value of 49 (9) % bound determined in the present study. The protein binding for the enantiomers of the other metabolites of hydroxychloroquine has not previously been investigated.

Implications of stereoselective plasma protein binding on the disposition of hydroxychloroquine in patients with rheumatoid arthritis

Enantioselectivity in the plasma protein binding of a chiral drug may result in stereoselective differences in drug action and/or disposition [Williams and Lee 1985; Levy and Boddy 1990]. The concentration of α_1-acid glycoprotein in blood has been shown to increase during different disease states [Piafsky 1980], including rheumatoid arthritis [Piafsky et al. 1978]. This acute phase protein is recognized as an important determinant of the plasma protein binding for basic drugs [Routledge 1986] and can provide a significant contribution to the intersubject variability in the plasma protein binding and disposition of some basic drugs [Piafsky and Borgå 1977]. During an episode of acute inflammation the concentrations of α_1-acid glycoprotein in blood are elevated and albumin concentrations are decreased [Zini et al. 1990; Skeith and Jamali 1991]. The data reported in Table 1 suggests that the ratio ((S)-/(R)-) of the protein binding of hydroxychloroquine enantiomers in a patient with rheumatoid arthritis would be decreased.

The total blood clearance of hydroxychloroquine calculated from blood concentration data is low [Tett et al. 1988]. For a low clearance drug, total blood clearance is expected to vary with variations in the fraction unbound [Gibaldi and Perrier 1982] suggesting that the variations in α_1-acid glycoprotein and albumin in inflammation would be reflected in changes in the total blood clearance of the enantiomers. However, changes in unbound concentrations are predicted to be transient and therefore of no clinical consequence for a slow acting drug such as hydroxychloroquine.

Tett et al. (1990b) have proposed that the very large volume of distribution of hydroxychloroquine is governed by tissue uptake by ion trapping in acidic components of cells rather than tissue protein binding. Since ion trapping depends on the pK_a of the drug and the pH of the trapping medium the process is not expected to be enantioselective.

Differences in the plasma protein binding of drug enantiomers may contribute to apparent enantioselective differences in renal excretion [Tucker and Lennard 1990; Levy and Boddy 1991]. Stereoselectivity in plasma protein binding affects the processes of glomerular filtration and passive tubular reabsorption, which will influence the net renal elimination of the enantiomers. Differences in the plasma protein binding of the enantiomers of hydroxychloroquine may, therefore, result in stereoselective differences in its renal clearance.

The pharmacodynamic implication of stereoselective plasma protein binding of hydroxychloroquine for patients with rheumatoid arthritis is unclear, as there is no published information on the relative activity of the enantiomers of hydroxychloroquine in this disease state.

References

Geary TG, Akood MA, Jensen JB (1983) Characteristics of chloroquine binding to glass and plastic. Am J Trop Med Hyg 32: 19–23

Gibaldi DA, Perrier D (1982) Pharmacokinetics, 2nd edn. Dekker, New York

Levy RH, Boddy AV (1991) Stereoselectivity in pharmacokinetics: a general theory. Pharm Res 8: 551–555

McLachlan AJ, Tett SE, Cutler DJ (1991) High-performance liquid chromatographic separation of the enantiomers of hydroxychloroquine and its major metabolites in biological fluids using α_1-acid glycoprotein stationary phase. J Chromatogr 570: 119–127

Miller DR, Khalil SRW, Nygan GA (1991) Steady-state pharmacokinetics of hydroxychloroquine in rheumatoid arthritis patients. Ann Pharmacother 25: 1302–1305

Ofori-Adjei D, Ericsson O, Lindstrom B, Sjöqvist F (1986) Protein binding of chloroquine enantiomers and desethylchloroquine. Br J Clin Pharmacol 22: 356–358

Piafsky KM, Borgä O (1977) Protein binding of basic drugs. II. Importance of α_1-acid glycoprotein for individual variation. Clin Pharmacol Ther 22: 545–549

Piafsky KM, Borgä O, Odar-Cederloe I, Johnsson C, Sjöqvist F (1978) Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma α_1-acid glycoprotein. New Eng J Med 299: 1435–1439

Piafsky KM (1980) Disease-induced changes in the plasma binding of basic drugs. Clin Pharmacokinet 5: 246–262
Routledge PA (1986) The plasma protein binding of basic drugs. Br J Clin Pharmacol 22: 499–506
Skeith KJ, Jamali F (1991) Clinical pharmacokinetics of drugs used in juvenile arthritis. Clin Pharmacokinet 21: 129–149
Tett SE, Cutler DJ, Day RO, Brown KF (1988) Dose-ranging study of the pharmacokinetics of hydroxychloroquine following intravenous administration to healthy volunteers. Br J Clin Pharmacol 26: 303–313
Tett SE, Cutler DJ, Day RO (1990a) Hydroxychloroquine concentrations and clinical effects in rheumatoid arthritis patients. Eur J Pharmacol 183: 1035
Tett SE, Cutler DJ, Day RO (1990b) Antimalarials in rheumatic diseases. In: Brooks PM (ed) Bailliere's clinical rheumatology. Slow-acting antirheumatic drugs and immunosuppressives. Baillière, Tindall, Sydney, pp 467–489
Tucker GT, Lennard MS (1990) Enantiomer specific pharmacokinetics. Pharmac Ther 45: 309–329
Walker O, Birkett DJ, Alván G, Gustafsson LL, Sjöqvist F (1983) Characterization of chloroquine plasma protein binding in man. Br J Clin Pharmacol 15: 375–377
Williams KM, Lee EJD (1985) Importance of drug enantiomers in clinical pharmacology. Drugs 30: 333–354
Yahya AM, McElnay JC, D'Arcy PF (1986) Investigation of chloroquine binding to plastic materials. Int J Pharm 34: 137–143
Zini R, Riant P, Barré J, Tillement J-P (1990) Disease induced variations in plasma protein levels: implications for drug dosage regimens (I). Clin Pharmacokinet 19: 147–159

Dr. D. J. Cutler
Department of Pharmacy
University of Sydney
NSW 2006
Australia