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Protein binding of chloroquine enantiomers and desethylchloroquine

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The protein binding of racemic chloroquine, its enantiomers and desethylchloroquine to plasma, purified human albumin, and α_1-acid glycoprotein (α_1-AGP) was determined by equilibrium dialysis. The binding was not concentration dependent. (+)-Chloroquine bound more to plasma (66.6 ± 1.9%) and albumin (45.9 ± 0.8%) than (−)-chloroquine (48.5 ± 2.4% and 35.3 ± 0.6%, respectively). These differences were statistically significant. (−)-Chloroquine bound more to α_1-AGP (47.5 ± 0.7%) than (+)-chloroquine (34.5 ± 0.5%). The binding of desethylchloroquine to α_1-AGP is higher than to albumin (38.9 ± 0.9% and 21.1 ± 0.4%, respectively.

Keywords protein binding stereoisomers chloroquine desethylchloroquine

Introduction

Chloroquine (CQ) is a racemic mixture of two enantiomers, (+)- and (−)-CQ. The activity of the two enantiomers against plasmodia was reported to be different *in vivo* (Haberkorn *et al.*, 1979) whereas no difference could be demonstrated in an *in vitro* system (Fu Sui *et al.*, 1986). This suggests that the disposition of CQ rather than the activity may be stereoselective. The major metabolite of CQ, desethylchloroquine (DCQ), is also active against CQ sensitive strains of *P. falciparum* (Aderounmu, 1984; Fu Sui *et al.*, 1986; Verdier *et al.*, 1984). The aim of the present study was to determine the binding of (±)-, (+)-, (−)-CQ and racemic DCQ to plasma, albumin and α_1-acid glycoprotein (α_1-AGP).

Methods

Heparinized blood from six healthy volunteers was centrifuged at 2000 *g* for 20 min and the plasma was stored at −20° C. Pooled plasma from the volunteers was used to assess interassay variation. Plasma from one volunteer was used to determine possible differences in binding of fresh and frozen plasma.

Phosphate buffer pH 7.4 was made from 4 g Na_2HPO_4, $2H_2O$, 0.775 g NaH_2PO_4, $1H_2O$ and 5.58 g NaCl in 1 l distilled water. The CQ enantiomers were synthesized (Blaschke *et al.*, 1978). Racemic CQ (ACO, Solna, Sweden), racemic DCQ (Sterling-Winthrop, Solna, Sweden) pure human albumin (Behringwerke AG, Marburg) and purified α_1-acid glycoprotein (Sigma, St Louis, USA) were used.

Equilibrium dialysis was performed using Teflon dialysis chambers and Technicon (Solna, Sweden) standard membranes. Experiments were done in duplicate using 500 µl of the buffer containing the drug against 500 µl of plasma. Equilibrium time was determined for the four drugs using 200 ng ml^{-1} of CQ and 20 ng ml^{-1} of DCQ and was found to be 2 h. Dialysis was carried out for 3 h in all experiments and at 21° C

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(Walker *et al.*, 1983). The binding of 100 ng ml^{-1} of (±)-, (+)-, (−)-CQ and 10 ng ml^{-1} of DCQ was determined in the plasma from the six volunteers. The effect of drug concentration on plasma protein binding was determined within the range of 25–400 ng ml^{-1} of CQ and 10–80 ng ml^{-1} of DCQ (the usual plasma concentration range after oral administration of 600 mg base of CQ). Specific binding of 200 ng ml^{-1} of (+)-, (−)-, (±)-CQ and 20 ng ml^{-1} of DCQ to 0.7 g l^{-1} of α_1-AGP and 40 g l^{-1} of albumin was determined in triplicate. The binding of 200 ng ml^{-1} of (±)-CQ to fresh and frozen plasma of one volunteer was studied in triplicate. The effect of DCQ concentration, 10–80 ng ml^{-1}, on the binding to albumin and α_1-AGP was also tested. CQ and DCQ were determined by h.p.l.c. (Alván *et al.*, 1982).

Protein binding was calculated using the formula $(C_p–C_t)/C_p \times 100$ where C_p is drug concentration in plasma and C_t the concentration in buffer after dialysis. Scatchard analysis was done on the binding to pooled plasma. The data were analysed using the paired *t*-test or unpaired Student's *t*-test where appropriate. Results are expressed as mean ± s.e. mean.

Results

The inter-assay variation was 6.4% expressed as coefficient of variation. There was no difference in binding between fresh and frozen plasma, 58.6 ± 0.8% and 59.4 ± 0.3%, respectively.

The protein binding data are shown in Table 1. The binding in plasma from the six healthy volunteers showed significant differences between the CQ species, (+)-CQ being more highly bound than (−)-CQ (66.6 ± 1.9% *vs* 48.5 ± 2.4%, $P < 0.001$). The binding of the racemate was significantly different from that of the enantiomers ((±) *vs* (+) $P < 0.05$, (±) *vs* (−) $P < 0.002$). Table 1 also shows that the binding to α_1-AGP and albumin is stereoselective.

Figure 1 shows the absence of an obvious relationship between drug concentration and extent of protein binding in pooled plasma over the concentration range tested.

There was no concentration dependency in the binding of DCQ to pooled plasma, albumin and α_1-AGP. The mean binding of DCQ at 20 ng ml^{-1} to α_1-AGP and albumin was 38.9 ± 0.9% and 21.1 ± 0.4% respectively.

The dissociation constant for (±)- and (+)-, (−)-CQ and DCQ was 640, 534, 1068 and 184 nmol l^{-1}. The total concentration of binding sites was 2.1, 2.2, 2.6 and 0.6 μmol l^{-1}, respectively.

Discussion

Steroisomers are different chemicals with distinct biological properties. In the event of one isomer being therapeutically inactive, it may potentially contribute to side effects. (+)-CQ has been shown to be more active against plasmodia in mice (Haberkorn *et al.*, 1979). Our own experience with human strains of CQ-sensitive and -resistant plasmodia *in vitro* indicate similar activity (Su *et al.*, 1986). However, the disposition of the stereoisomers may be different. Our results for both % binding and dissociation constants, indicate that (+)-CQ binds more to plasma than (−)-CQ. The value for binding of (±)-CQ to pooled plasma is in agreement with published data (Walker *et al.*, 1983). The hierarchy of binding to albumin was similar to plasma. We found that (−)-CQ binds more strongly than (+)-CQ to α_1-AGP. The higher binding of (+)-CQ in plasma may be explained by the hypothesis that it preferentially binds to other proteins in plasma, e.g. lipoproteins and/or globulins.

The major metabolite of CQ, DCQ, is known to be active against CQ sensitive strains of plasmodium (Aderounmu, 1984; Verdier *et al.*, 1984; Su *et al.*, 1986) but its protein binding characteristics have not been reported. It is bound to the same extent as CQ in plasma and the binding is not concentration dependent. Its binding to α_1-AGP is greater than to albumin, 37.2 ± 0.7% and 23.1 ± 0.9%, respectively.

Table 1 Per cent binding of (±)-CQ, (+)-CQ, (−)-CQ and DCQ to plasma from healthy volunteers, α_1-AGP and albumin (mean ± s.e. mean).

	Healthy volunteers (n = 6)	α_1*-AGP (0.7 g l^{-1})*	*Albumin (40 g l^{-1})*
(±)-CQ	58.8 ± 1.8	39.3 ± 1.0	41.8 ± 0.6
(+)-CQ	66.6 ± 1.9	34.5 ± 0.5	45.9 ± 0.8
(−)-CQ	48.5 ± 2.4	47.5 ± 0.7	35.3 ± 0.6
DCQ	56.6 ± 2.7	38.9 ± 0.9	21.1 ± 0.4

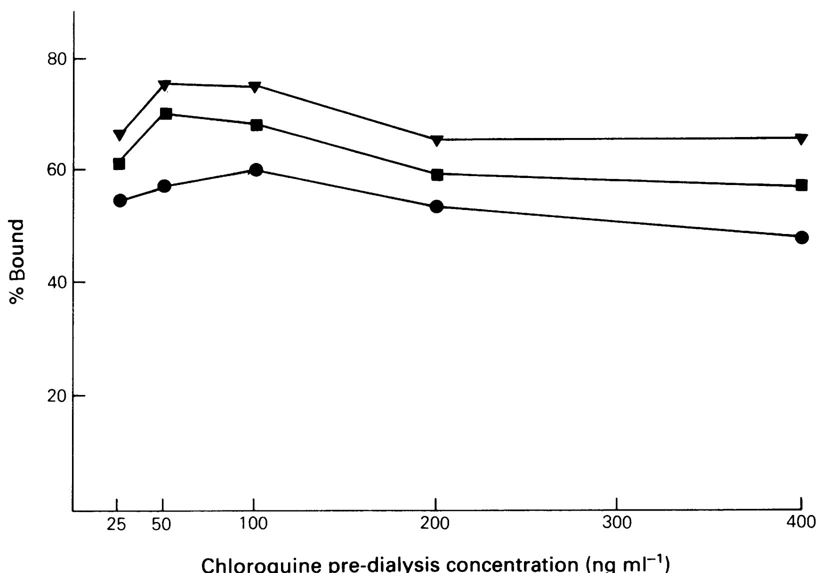

Figure 1 Effect of drug concentration on the protein binding of racemic CQ (■), (+)-CQ (▼) and (−)-CQ (●) to pooled plasma.

We have shown that the binding of CQ to plasma, α_1-AGP and albumin is stereoselective. The clinical significance of this is presently not apparent. However the information should prove useful in future work relating to stereoselective disposition of CQ.

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