Br. J. clin. Pharmac. (1987), **24**, 769–774

Differences in the binding of quinine and quinidine to plasma proteins

GEORGE W. MIHALY, MICHAEL S. CHING, MARGARET B. KLEJN, JOHN PAULL[1] & RICHARD A. SMALLWOOD

Department of Medicine, University of Melbourne, Austin Hospital, Melbourne, Victoria 3084, Australia and [1]Royal Women's Hospital, Grattan Street, Melbourne, Victoria 3052, Australia

1 Little is known about the comparative plasma protein binding of the antimalarial agents quinine (QN) and its isomer quinidine (QD). We have examined the *in vitro* binding of QN and QD to albumin, α_1-acid glycoprotein, normal human plasma, and maternal and foetal umbilical cord plasma.

2 QN was more avidly bound than QD, and binding of both drugs was substantially higher to α_1-acid glycoprotein than to albumin, indicating that α_1-acid glycoprotein is the more important binding protein.

3 Protein and drug concentration dependent binding was evident for both QN and QD. The unbound fraction of both drugs fell with increasing albumin (10 to 60 g l^{-1}) and α_1-acid glycoprotein (0.5 to 2.0 g l^{-1}) concentration, and there was a marked increase in unbound fraction of QN (6 to 19%) and QD (13 to 36%) in human plasma when drug concentrations were increased over the antimalarial therapeutic range (0.5 to 10 mg l^{-1}).

4 In human volunteer plasma, the unbound fractions of QN and QD were 7.5 ± 2.2% and 12.3 ± 2.3% respectively, whilst the unbound fractions for both drugs were significantly higher in maternal plasma (QN = 13.0 ± 5.4%, QD = 18.3 ± 2.5%) and significantly higher still in foetal umbilical cord plasma (QN = 25.7 ± 10%, QD = 35 ± 5.3%).

Keywords quinine quinidine antimalarial agents protein binding

Introduction

The emergence of resistance by the malaria parasite to synthetic antimalarial drugs has prompted a reappraisal of the cinchona alkaloids, used singly or in combination with other antimalarials, for the treatment of malaria (Phillips *et al.*, 1985; White *et al.*, 1982; Looareesuwan *et al.*, 1985; White, 1985). In South East Asia, for example, severe infection in obstetric patients is now treated with quinine, which has traditionally been the preferred antimalarial amongst these alkaloids (Looareesuwan *et al.*, 1985; Phillips *et al.*, 1986). Its diastereoisomer quinidine (Figure 1), which is probably even more potent than quinine (White, 1985), is also gaining wide use (Phillips *et al.*, 1985).

The binding of quinine and quinidine to human plasma is reported to be greater than 75% (Ochs *et al.*, 1980; Silamut *et al.*, 1985). While quinine and quinidine have both been shown to bind to albumin, quinidine has also been reported to bind to α_1-acid glycoprotein (AAG) (Ochs *et al.*, 1980). However, a detailed comparison of the binding characteristics of these diastereoisomers to different plasma

Correspondence: Dr George Mihaly, Department of Medicine, Austin Hospital, Melbourne, Victoria 3084, Australia

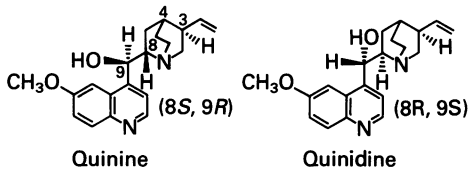

Figure 1 Structural formulae of quinine and quinidine, showing asymmetric centres at positions 8 and 9.

proteins has not been undertaken. In particular, the effect on binding (and hence on the *pharmacologically active*, unbound drug concentration) of changes in drug or plasma protein concentrations is not well defined. This is of some interest, since during malaria infection plasma albumin concentration falls by up to 25%, while that of acute phase globulins, including AAG, may rise by 50% (Silamut *et al.*, 1985).

The present study examines *in vitro* the effects of changing drug and protein concentrations on binding, and in view of the increasing obstetric use of these drugs, compares binding in simultaneously-collected maternal and mixed umbilical cord plasma.

Methods

Materials

Quinine hydrochloride and quinidine hydrochloride monohydrate were obtained from Sigma Chemical Co. (St Louis, MO). Bovine serum albumin (BSA) and α_1-acid glycoprotein (Cohn fraction V1–1B, batch no. 1004) were supplied by Commonwealth Serum Laboratories (Melbourne, Australia). Cellulose dialysis membrane, type 20 was obtained from Union Carbide Corp. (NY, USA).

Plasma samples

Healthy volunteers Blood (20 ml) was obtained by venepuncture from 15 healthy adults (eight female and seven male), aged 19–39 years (mean age for females = 27, mean age for males = 29). The blood was collected into lithium heparin tubes and after centrifugation (3000 rev min^{-1}, 10 min) the plasma was separated and transferred into individual plain plastic tubes. Half the plasma from each subject was spiked to 1 mg l^{-1} with quinine, and half with quinidine (1 mg l^{-1}). In each case concentrated aqueous stock solutions were used (100 mg l^{-1}). The plasma protein binding in each sample was then determined by equilibrium dialysis.

Obstetric patients Blood (20 ml) was collected by venepuncture from four women immediately post partum. None of the four patients had received any medication during delivery. Mixed cord blood (3–10 ml) was collected by syringe and as with maternal samples, placed in lithium heparin tubes. The resultant plasma was treated in the same way as that collected from healthy subjects.

Binding studies; effect of varying protein and drug concentration

(a) The protein binding of quinine and quinidine was examined by equilibrium dialysis, in Sorensen's buffer (pH 7.4), of varying protein concentration (BSA = 10–60 g l^{-1}; AAG = 0.5–2 g l^{-1}) with drug concentrations held constant (1 mg l^{-1}) and in buffer solutions of varying drug concentrations (0–10 mg l^{-1}), but protein concentrations held constant (BSA = 20 g l^{-1} and AAG = 1 g l^{-1}).
(b) Binding was determined in pooled plasma from healthy volunteers with drug concentrations varying from 0–10 mg l^{-1}.

Equilibrium dialysis

Protein solutions and samples of plasma (2 ml) were dialyzed against drug free Sorensen's buffer (2 ml; pH 7.4) in perspex chambers separated by cellulose dialysis membrane (Chignell, 1977). Dialysis cells were incubated at 37° C for 24 h. Equilibration was previously determined to have been completed by 18 to 20 h. After dialysis, the plasma and buffer pH were checked in selected samples and were found to be within ± 0.1 pH unit of the predialysis value. The buffer was examined for protein contamination using Albustix (Ames Division, Miles Labs., Australia; sensitivity = 0.05 g l^{-1}). The unbound fraction (f_u) was determined by the ratio of drug concentration in the 'buffer' side to that in the 'sample' side.

Analysis

Concentrations of quinine and quinidine in samples of plasma, protein solution and buffer solutions were determined by a selective and

sensitive high performance liquid chromatographic method (Mihaly *et al.*, 1987). Concentrations of albumin and AAG in plasma samples obtained from volunteers, obstetric patients and umbilical cord were determined in duplicate using a commercially available radioimmunodiffusion assay kit that has an accuracy of ± 15% (Nor-Partigen, Behringwerke, AG, Germany).

Calculations

Results are presented as mean ± s.d. in the text and as mean ± s.e. mean graphically. Statistical comparisons were made using paired Student's *t*-test for paired observations and unpaired Student's *t*-test for unpaired observations, accepting $P < 0.05$ as significant.

Results

The binding of quinine was significantly greater than that of quinidine in all experiments (Figures 2 and 3; Table 1). Binding of both drugs was substantially higher to AAG than to albumin at concentrations of these proteins which encompassed the expected range in human plasma, indicating that AAG is the more important binding protein in plasma.

Table 1 Quinine and quinidine binding in human plasma

Subjects	% *unbound* (f_u) *Quinine* (n)	*Quinidine* (n)
Male volunteers	7.3 ± 2.6 (7)	12.1 ± 2.1 (7)
Female volunteers	7.8 ± 1.9 (8)	12.4 ± 2.6 (8)
Mothers	13.0 ± 5.4 (4)	18.3 ± 2.5 (3)
Foetus	25.7 ± 10.0 (4)	35.0 ± 5.3 (3*)

*Insufficient foetal plasma in one out of four sets (Quinine and quinidine spiked to 1 mg l^{-1}).

Protein concentration dependent binding of both drugs to both AAG and albumin was demonstrated (Figure 2). Increasing albumin concentration from 10 to 60 g l^{-1} decreased f_u of quinine from 0.63 ± 0.02 to 0.22 ± 0.02, and of quinidine from 0.72 ± 0.08 to 0.31 ± 0.03. Similarly over an AAG concentration range of 0.5–2.0 g l^{-1}, quinine f_u fell from 0.13 ± 0.05 to 0.02 ± 0.01, and that of quinidine from 0.24 ± 0.02 to 0.05 ± 0.02.

Both drugs showed drug concentration dependent binding in human plasma, and in solutions of AAG and albumin (Figure 3). In plasma ([albumin] = 33.9 g l^{-1}; [AAG] = 0.9 g l^{-1}), quinine f_u increased 3-fold from 0.06 ± 0.01 to 0.19 ± 0.02, while quinidine f_u increased from

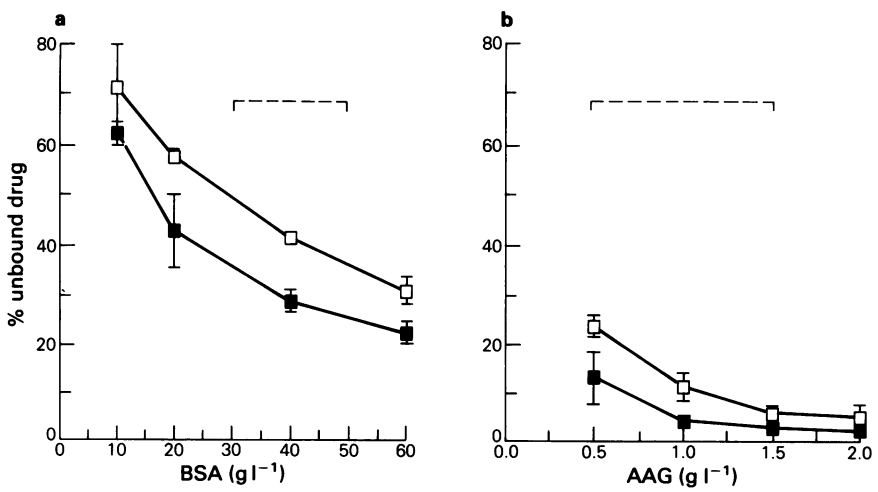

Figure 2 Effect of bovine serum albumin concentration (a) and α_1-acid glycoprotein concentration (b) on the unbound fraction of quinine (■) and quinidine (□). The normal range of these protein concentrations, in plasma from healthy human subjects, is also shown by the dotted line.

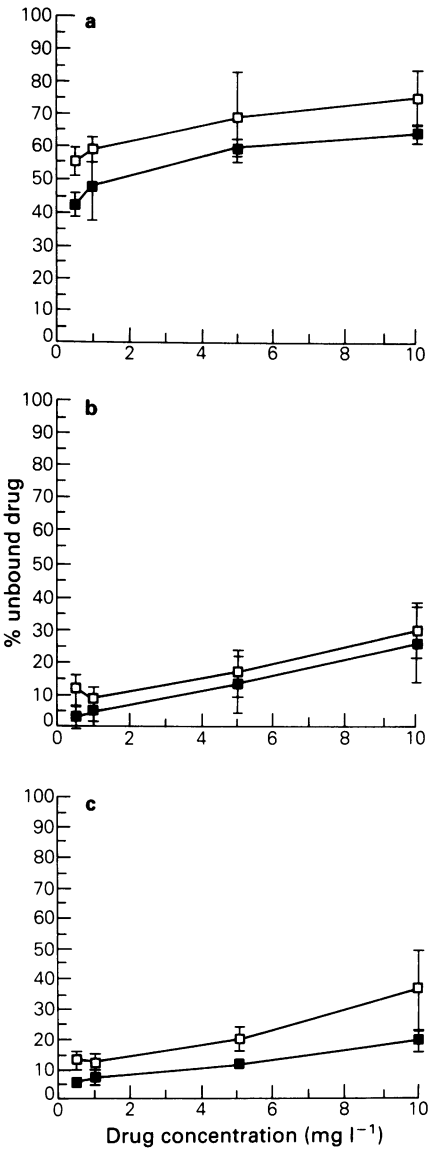

Figure 3 Effect of quinine (■) and quinidine (□) concentration on the unbound fraction of drug in solutions of bovine serum albumin (a) and α_1-acid glycoprotein (b), and in human plasma (c).

0.13 ± 0.01 to 0.36 ± 0.08, when drug concentrations were increased from 0.5 to 10 mg l^{-1}. Similar changes were seen with increasing drug concentrations in solutions containing AAG or albumin at constant concentrations (1.0 and 20 g l^{-1} respectively) (Figure 3). However, due to the very high binding of quinine to AAG at low drug concentrations ($f_u = 0.03 \pm 0.03$ at 0.5 mg l^{-1}), the increase in f_u to 0.26 as drug concentration was increased to 10 mg l^{-1} represented much the highest *percent* increase in f_u found (870% vs 50%).

Table 2 Protein concentration in the plasma of healthy volunteers, pregnant women at term and foetal cord plasma

		Protein concentration (g l^{-1})	
Subjects	n	*Albumin*	*AAG*
Males	7	48.9 ± 2.7	0.736 ± 0.122
Females	8	47.1 ± 4.5	0.677 ± 0.099
Maternal	3	34.9 ± 3.4	0.629 ± 0.080
Foetal	3	44.0 ± 2.1	0.309 ± 0.110

In mothers at the time of delivery, f_u for both quinine and quinidine was approximately two-fold higher than in non-pregnant women (Table 1). This lower binding was associated with reduced plasma concentrations of albumin and AAG in mothers at term (Table 2). The f_u for both drugs was two-fold higher again in cord plasma, and this seemed to reflect significantly lower concentrations of AAG in particular (Tables 1 and 2).

Discussion

Despite the widespread use of quinine and quinidine over many years, there have been few studies of the protein binding of these two cinchona alkaloids. The binding of quinidine has been examined in a number of studies in normal volunteers and cardiological patients, and f_u was found to range from 0.05 to 0.25 (Ochs *et al.*, 1980; for review). A recent study in patients with cerebral malaria found that f_u of quinine during the illness was lower than that in convalescent patients (0.07 ± 0.04 vs 0.11 ± 0.06, Silamut *et al.*, 1985).

In the present study we have directly compared the binding characteristics of these two diastereoisomers in buffer solutions of varying protein content, and in plasma from healthy volunteers, pregnant women, and umbilical cord plasma. The protein binding of quinine was consistently greater than that of quinidine, so that at comparable total concentrations the *pharmacologically active* unbound concentration of quinine is appreciably less than that of quinidine (Figures 2 and 3). As with many other

basic drugs (e.g. propranolol, alprenolol, lignocaine, etidocaine (Piafsky, 1983; Routledge, 1986)) the high binding (80–90% or more) of both these cinchona alkaloids is likely to be largely accounted for by the avid binding to AAG (Figure 2).

The protein concentration dependent binding of these drugs (Figure 2) may well be of clinical importance. During malaria infection, acute phase globulin (and therefore presumably AAG) concentrations have been shown to rise by up to one third (Silamut *et al.*, 1985), although there is often a compensating fall in albumin concentration of 25–30% (Silamut *et al.*, 1985). This makes prediction of binding trends in the individual patient uncertain. Nonetheless, in the study of Silamut *et al.* (1985) there was a consistent and clear-cut fall in quinine f_u during cerebral malaria, which suggests that the presumed rise in AAG concentration outweighs any fall in albumin concentration. A further element of uncertainty in adjusting dosage to achieve a predictable concentration of unbound drug is provided by the demonstration that the clearance of unbound quinine may be reduced by two thirds during cerebral malaria (White *et al.*, 1982). The net effect of these binding and clearance changes on the likelihood of quinine toxicity is uncertain, and so there have been predictions of increased (Hall, 1985) and decreased (Warrell *et al.*, 1985) quinine toxicity in cerebral malaria patients. Similar difficulties would presumably be encountered in considering quinidine dosage, although there have been no studies of quinidine binding or clearance in malaria-infected patients.

The present study demonstrated drug concentration dependent binding of both drugs over the range of drug levels that would be encountered during therapy (0–10 mg l^{-1}). Silamut *et al.* (1985) did not find any change in quinine f_u with increasing total drug concentration, and Edwards *et al.* (1984) found similarly that quinidine f_u did not change at total concentrations between 1 and 5 mg l^{-1}. Although the reason for the disparity between our present findings and those of the earlier studies is not apparent, it may in part be related to the fact that these earlier studies employed fluorescent assay methods which Edstein *et al.* (1983) reported to be non-selective, and to the fact that Edwards *et al.* (1984) did not study binding at a concentration range appropriate for antimalarial treatment (5–10 mg l^{-1}; Phillips *et al*, 1985).

In pregnant women, f_u of both drugs was nearly two-fold greater than in healthy adult volunteers (Table 1). This reduced binding reflects the lower plasma protein concentrations found in the latter part of pregnancy (Cummings, 1983; Perucca & Crema, 1982) and the observed protein concentration dependent binding (Figures 2 and 3). Similar changes have been shown for a number of other drugs in near-term pregnant women (Herngren *et al.*, 1983; Dean *et al.*, 1980). It is well known that reduced maternal drug binding, as well as pregnancy itself, can influence drug clearance in the mother; the effect will vary according to the organ and mechanism of elimination (Mihaly & Morgan, 1984; Routledge, 1986). Since the reduced drug binding to maternal plasma may be associated with a change in maternal clearance, this generally lower drug binding indicates that, for highly bound drugs, the unbound as well as the total plasma concentration may need to be monitored in full term pregnant women. A recent study in Thai patients, Phillips *et al.* (1986) found the *relative* foetal exposure to quinine (i.e. the ratio of foetal to maternal total drug concentrations) to be somewhat variable. However, since drug binding was not examined, absolute foetal exposure (i.e. the unbound concentration of drug in foetal plasma (Mihaly & Morgan, 1984) could not be established.

According to Mihaly & Morgan (1984), absolute foetal exposure is largely determined by the unbound drug concentration in maternal plasma. Since quinidine is less highly bound in maternal plasma than quinine, for the same total drug concentration in the mother, the absolute foetal exposure of the foetus will be greater for quinidine than quinine by a factor of 1.5 to 2. Differences in the binding between these diastereomers may therefore be one of several factors in deciding which is the safer drug to use in the antimalarial treatment of obstetric patients.

This work was supported by the National Health and Medical Research Council of Australia. We wish to acknowledge the valued clerical assistance of Ms Jane Bell.

References

Chignell, C. F. (1977). Protein binding. In *Drug fate and metabolism*, eds. Garrett, E. R. & Hirtz, J. L. Vol. 1, pp. 184–228; New York: Marcell Dekker.

Cummings, A. J., (1983). A survey of pharmacokinetic data from pregnant women. *Clin. Pharmacokin.*, **8**, 344–354.

Dean, M., Stock, B., Patterson, R. J. & Levy, G. (1980). Serum protein binding of drugs during and

after pregnancy in humans. *Clin. Pharmac. Ther.*, **28**, 253–261.

Edstein, M., Stace, J. & Shann, F. (1983). Quantification of quinine in human serum by HPLC. *J. Chromatogr.*, **278**, 445–451.

Edwards, D. J., Axelson, J. E., Slaughter, R. L., Elvin, A. T. & Lalka, D. (1984). Factors affecting quinidine protein binding in humans. *J. pharm. Sci.*, **73**, 1264–1267.

Hall, A. (1985). Dangers of high dose quinine and overhydration in severe malaria. *Lancet*, **i**, 1453.

Herngren, L., Ehrnebo, M. & Boreus, L. O. (1983). Drug binding to plasma proteins during human pregnancy and in the perinatal period. *Dev. Pharmac. Ther.*, **6**, 110–124.

Looareesuwan, S., White, N. J., Karbwang, J., Turner, R. C., Phillips, R. E., Kietinun, S., Rackow, C. & Warrell, D. A. (1985). Quinine and severe falciparum malaria in late pregnancy. *Lancet*, **ii**, 4–7.

Mihaly, G. W., Hyman, K. M., Smallwood, R. A. & Hardy, K. J. (1987). High performance liquid chromatographic analysis of quinine and its diastereoisomer quinidine. *J. Chromatogr.*, **415**, 177–182.

Mihaly, G. W. & Morgan, D. J. (1984). Placental drug transfer: effect of gestational age and species. *Pharmac. Ther.*, **23**, 253–266.

Ochs, H. R., Greenblatt, D. J. & Woo, E. (1980). Clinical pharmacokinetics of quinidine. *Clin. Pharmacokin.*, **5**, 150–168.

Perucca, E. & Crema, A. (1982). Plasma protein binding of drugs in pregnancy. *Clin. Pharmacokin.*, **7**, 336–352.

Phillips, R. E., Looareesuwan, S., Silamut, K., Kietinun, S. & Warrell, D. A. (1986). Quinine pharmacokinetics and toxicity in pregnant and lactating women with falciparum malaria. *Br. J. clin. Pharmac.*, **21**, 677–683.

Phillips, R. E., Warrell, D. A., White, N. J., Looareesuwan, M. D. & Karbwang, J. (1985). Intravenous quinidine for the treatment of severe falciparum malaria. *New Engl. J. Med.*, **312**, 1273–1278.

Piafsky, K. M., (1983). Disease-induced changes in the plasma binding of basic drugs. In *Handbook of clinical pharmacokinetics*, eds. Gibaldi, M. & Prescott, L. Sec. II, pp. 70–88. Sydney: ADIS Health Science Press.

Routledge, P. A. (1986). The plasma protein binding of basic drugs. *Br. J. clin. Pharmac.*, **22**, 499–506.

Silamut, K., White, N., Looareesuwan, S. & Warrell, D. J. (1985). Binding of quinine to plasma proteins in falciparum malaria. *Am. J. Trop. Med. Hyg.*, **34**, 681–686.

Warrell, D. A., White, N. J., Looareesuwan, S., Phillips, R. E., Warrell, M. J., Bunnag, D. & Harinasuta, K. T. (1985). Dangers of high dose quinine and overhydration in severe malaria – A reply. *Lancet*, **i**, 1453–1454.

White, N. (1985). Clinical pharmacokinetics of antimalarial drugs. *Clin. Pharmacokin.*, **10**, 187–215.

White, N., Looareesuwan, S., Warrell, D. A., Warrell, M. J., Bunnag, D. & Harinasute, T. (1982). Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria. *Am. J. Med.*, **73**, 564–572.

(Received 8 April 1987, accepted 5 August 1987)