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Plasma Protein Binding of Quinine: Binding to Human Serum Albumin, α_1-Acid Glycoprotein and Plasma from Patients with Malaria

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Abstract—The binding of quinine to human serum albumin (HSA), α_1-acid glycoprotein (AAG) and plasma obtained from healthy subjects (10 caucasians and 15 Thais) and from Thai patients with falciparum malaria (n = 20) has been investigated. In healthy volunteers, plasma protein binding expressed as the percentage of unbound quinine was 7·9–31·0% (69–92·1% bound). The mean percentage of unbound quinine found with essentially fatty acid-free HSA (40 g L^{-1}) was 65·4 ± 1·5% (mean ± s.d.) and was comparable with the value (66·3 ± 3·8%, mean ± s.d.) for Fraction V HSA (40 g L^{-1}). This suggests that fatty acids do not influence the plasma protein binding of quinine. Binding of quinine to 0·7 g L^{-1} AAG was high (mean unbound 61·0 ± 5·0%), indicating that quinine is bound primarily to AAG and albumin, although other plasma proteins such as lipoproteins may be involved. The mean percentage of unbound quinine was slightly less in caucasians (14·8 ± 6·7% unbound), compared with healthy Thai subjects (17·0 ± 6·7% unbound). The higher binding of quinine in caucasian subjects was associated with a higher plasma AAG concentration observed in caucasians. Mean percentage of unbound quinine was significantly lower in Thai patients with malaria (10·9 ± 4·0%) than in the healthy Thai subjects. The increase in the extent of quinine binding corresponded with the increase in the acute-phase reactant protein, AAG in the patients with malaria. Overall, when the data were combined there was a significant correlation ($r = 0·846$, $P < 0·005$) between the binding ratio (bound/unbound) of quinine and the plasma AAG concentration. This suggests that plasma AAG concentration may serve as a useful index to predict alterations in quinine binding. Although quinine is bound to albumin, it was not bound to either site I or site II on HSA as indicated from equilibrium dialysis and fluorescent probe displacement studies. Binding displacement studies revealed that there was no marked displacement of quinine by a variety of highly bound acidic and basic drugs, including other antimalarial drugs at their therapeutic concentrations.

The importance of binding of drugs to plasma proteins has been reviewed (Koch-Weser & Sellers 1976; Vallner 1977; Routledge 1986). Most of the binding of acidic drugs can be explained by albumin association. However, this is not the case with basic drugs. Although some binding to albumin occurs, it is now known that basic drugs bind primarily to α_1-acid glycoprotein (AAG) or to lipoprotein fractions rather than to albumin (Piafsky 1980; Routledge 1986). Quinine is one of the oldest drugs in the pharmacopoeia. While synthetic antimalarial drugs have largely replaced quinine, the emergence of *Plasmodium falciparum* resistant to chloroquine in many endemic malaria areas, has necessitated its continued therapeutic use (White 1985). The plasma protein binding of quinine is reported to be greater than 75% (Mihaly et al 1987; Mansor et al 1991; Silamut et al 1985). In-vitro studies have demonstrated that quinine is substantially more highly bound to AAG than to albumin (Mihaly et al 1987). Binding of quinine was increased during malaria infection and Silamut et al (1985) suspected that the increase in quinine binding observed in the patients with malaria was likely to be due to a marked rise in plasma AAG concentration; concentrations of AAG were not measured in that study. At the time we commenced our study, there was no supporting evidence. Therefore, we have investigated the in-vitro binding of quinine to human serum albumin (HSA) and AAG. The binding of quinine was also determined in plasma samples from healthy volunteers (caucasian and Thai) and patients with falciparum malaria. The role of AAG and albumin in the binding of quinine has been investigated.

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Materials and Methods

Materials

Quinine bisulphate was kindly supplied by Kimia Pharma, Indonesia. [^{14}C]Warfarin (15 μCi μmol^{-1}; Amersham, UK) and [^{14}C]diazepam (57 μCi μmol^{-1}; Amersham, UK) were gifts from Professor D. J. Birkett, Flinders Medical Centre, Australia. The radiolabelled drugs were >98% pure as determined by thin layer chromatography. Human serum albumin (HSA), essentially fatty acid free, Fraction V, α_1-acid glycoprotein (AAG), 5-dimethylaminonaphthalene-1-sulphonamide (DNSA) and dansylsarcosine were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of analytical grade.

Protein solutions, subjects and patients

HSA and AAG solutions were prepared in 0·1 M NaH_2PO_4 buffer (pH 7·4) containing 0·9% (w/v) NaCl. The mol. wt of albumin was taken as 66 500 Da. Binding studies were also carried out using pooled blood bank plasma and plasma samples collected from 10 caucasian healthy subjects (3 females and 7 males). They ranged in age from 19 to 47 years (mean ± s.d., 31 ± 8 years). Effects of malaria on quinine binding was investigated by determining drug binding in plasma obtained from 20 Thai patients with *Plasmodium*

falciparum malaria (8 females and 12 males), aged 20 to 55 years with a mean age of 35 ± 10 years. A group of 15 healthy Thai volunteers (5 females and 10 males) between 18 and 50 years of age (mean ± s.d., 29 ± 8 years) served as a control for comparison. All healthy volunteers were determined to be healthy after medical histories were obtained and physical examinations were performed, and there was no biochemical evidence of renal or hepatic dysfunction. None of the subjects took any drugs (except for vitamin tablets) for at least 2 weeks. The plasma samples from patients with falciparum malaria were collected upon their admission but before drug treatment. Of 20 patients participating in the study, 16 were patients with uncomplicated malaria and 4 were patients with cerebral malaria. None of the patients had renal dysfunction. Patients who were known to take any antimalarial drugs and other highly protein-bound drugs were excluded. After centrifugation of the blood sample, plasma was separated and kept frozen at −20°C for subsequent analysis of plasma proteins and quinine binding. The study was approved by the local ethics committees of hospitals in Thailand.

Albumin and AAG measurement

Albumin concentrations were measured by the bromocresol green method and total protein concentrations using the Biuret reagent. Plasma concentrations of AAG were determined by radial immunodiffusion using a commercial kit (Nor-Partigen, Behring Diagnostics, CA, USA). Plasma free fatty acid concentrations were measured by the method of Duncombe (1964).

Protein binding experiments

All experiments were performed using a 0·1 M sodium phosphate buffer (pH 7·4) containing 0·9% (w/v) NaCl. Binding of quinine was measured by equilibrium dialysis at 37°C. Protein solution or plasma (1 mL) was dialysed against 0·1 M isotonic phosphate buffer (pH 7·4) containing quinine at an initial concentration of 3 μg mL^{-1} for 3 h. Dialysis was performed using a Dianorm apparatus (Diachema AG, Zurich, Switzerland) with 1 mL capacity cells and Spectrapor 2 membrane tubing (Spectrum Medical Industries, CA, USA). Our preliminary experiments have shown that quinine equilibrium was achieved within 3 h. No significant binding of drug to the dialysis membrane and cell walls was observed. Volume shifts were negligible. After dialysis, aliquots from each side of the cells were analysed for quinine concentrations by a selective and sensitive HPLC procedure (Zoest et al 1990). Binding of [^{14}C]warfarin and [^{14}C]diazepam in the presence of quinine was also carried out by equilibrium dialysis as described above. After dialysis, 0·5 mL samples from both sides of the cells were mixed with 5 mL of PCS (a complete Phase Combining System, the Radiochemical Centre, Amersham, UK) and radioactivity was determined by liquid scintillation counting with external standardization. Each measurement of binding was performed with at least two replications.

Displacement of quinine by other antimalarial drugs and other various drugs was studied by equilibrium dialysis using drug concentrations reported to occur at therapeutic doses. Unless otherwise stated, the concentration of quinine used in binding and displacement experiments was 3 μg mL^{-1}.

In order to identify the binding sites of quinine on HSA, displacement studies with fluorescent probes were performed. The displacement of fluorescent probes specific for sites I and II, i.e. DNSA (site I) and dansylsarcosine (site II), by quinine and reference compounds was measured as previously described (Sudlow et al 1975). Fluorescence measurements were made at room temperature (22°C) using a Shimadzu RF540 fluorescence spectrophotometer (Shimadzu, Kyoto, Japan).

Statistical analysis

Results are reported as mean ± s.d. When appropriate, statistical analysis was performed using Student's *t*-test. The relationship between plasma protein concentrations and binding ratio (number of moles bound divided by the number of moles unbound) of quinine was examined by linear regression analysis. The limit of significance was taken as $P < 0.05$.

Results

Binding of quinine in albumin, AAG solutions, and plasma

The extent of quinine binding was examined with two preparations of HSA, AAG solution and various samples of plasma (Table 1). Quinine was 69–92% bound in human plasma. The degree of binding observed in HSA and AAG solutions was similar, suggesting that quinine binds to both albumin and AAG. There was no significant difference between the percentage of unbound quinine in the fatty acid-free HSA and Fraction V HSA (1·9 mol fatty acid per mol HSA). This suggests that fatty acids may have no influence on the binding of quinine.

Effects of heparin, storage conditions, quinine concentration and temperature

Quinine binding was similar in serum and heparinized plasma (% unbound: serum, 10·0 ± 3·0 (n = 6); heparinized plasma, 10·5 ± 3·3 (n = 6)). The binding of quinine was not altered by storage of plasma or serum at −20°C for up to 2 months. Binding of quinine over the initial concentration range of 0·5–10 μg mL^{-1} was studied with normal plasma. The results showed that there was a linear relationship between percentage of unbound quinine and total plasma drug concentration over the concentration range studied. The unbound quinine in human plasma was 6·4% at a quinine concentration of 0·5 μg mL^{-1} and increased to 23·4% at a drug concentration of 10 μg mL^{-1}. The percentage of unbound quinine was significantly decreased ($P < 0.05$) when the binding experiment was performed at 22°C (% unbound: 16·7 ± 1·4 (n = 6)) as compared with those values obtained at 37°C (% unbound: 20·4 ± 1·3 (n = 6)). This indicates that the binding of quinine is decreased with increasing temperature.

Binding of quinine to plasma proteins in normal volunteers and patients with malaria

Table 1 lists the data on quinine binding in three different groups of subjects, i.e. healthy caucasian, healthy Thai subjects, and Thai patients with falciparum malaria. There was a sex and age match with these three groups. The percentage of unbound quinine was slightly less in cauca-

Table 1. Quinine binding to human serum albumin (HSA), α_1-acid glycoprotein (AAG) and plasma from healthy volunteers and patients with falciparum malaria.

Sample	Unbound quinine (%)	Albumin (g L^{-1})	AAG (g L^{-1})	Total protein (g L^{-1})
Essentially fatty acid-free HSA (40 g L^{-1}) (n = 5)	65·4 ± 1·5	40	—	40
Fraction V HSA (40 g L^{-1}) (n = 5)	66·3 ± 3·8	40	—	40
AAG (0·7 g L^{-1}) (n = 6)	61·0 ± 5·0	—	0·7	0·7
Pooled blood bank plasma (n = 6)	20·7 ± 1·3	39	0·75	63
Plasma samples from 10 healthy caucasians	14·8 ± 6·7 (range 7·9–31·0)	43·8 ± 4·2	0·86 ± 0·16*	72 ± 6
Plasma samples from 15 healthy Thai subjects	17·0 ± 5·3 (range 9·2–29·5)	40·2 ± 3·3	0·64 ± 0·19	68 ± 5
Plasma samples from 20 Thai patients with falciparum malaria	10·9 ± 4·0** (range 5·5–22·1)	36·5 ± 4·0*	1·23 ± 0·48*	66 ± 7

* $P < 0·05$, ** $P < 0·001$ compared with the corresponding value observed in healthy Thai subjects.

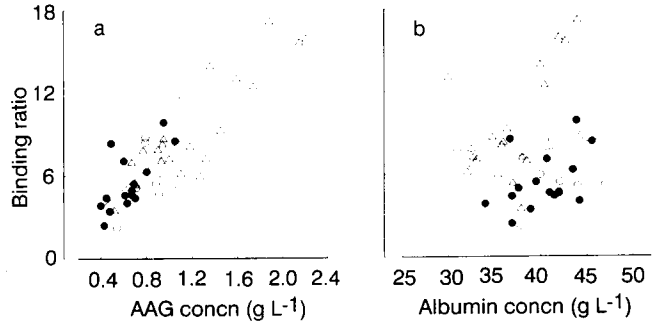

FIG. 1. Binding ratio (B/F) of quinine in relation to plasma α_1-acid glycoprotein, AAG (a) and plasma albumin (b) concentrations. B and F represent the molar concentrations of bound and unbound (free) quinine, respectively. The binding of quinine was measured at an initial total concentration of 3 μg mL^{-1}. The least-squares regression lines are: a, $y = 0·83 + 7·05x$ ($r = 0·846$, $P < 0·005$); and b, $y = 2·04 + 0·14x$ ($r = 0·183$, $P > 0·05$), ○ Caucasians (n = 10), ● healthy Thai subjects (n = 15), △ Thai patients with falciparum malaria (n = 20).

sians, compared with healthy Thai subjects but this difference was not significant (Table 1). The plasma AAG concentration was approximately 34% higher in samples from caucasian subjects (0·86 ± 0·16 vs 0·64 ± 0·19 g L^{-1}, $P < 0·05$). The plasma albumin concentration in samples from caucasians was slightly higher than in those from Thai subjects, but this difference was not statistically significant.

The plasma protein binding of quinine was determined in 20 Thai patients with falciparum malaria before drug therapy. Mean percentage of unbound quinine in the patients with malaria was significantly less than in the healthy Thai volunteers (Table 1, % unbound: 10·9 ± 4·0 vs 17·0 ± 5·3, $P < 0·001$). These results indicate that quinine binding is greater in patients with malaria, compared with healthy subjects. Mean plasma AAG concentration in the patients with malaria was significantly higher ($P < 0·05$) than that in the healthy Thai controls (Table 1). Serum albumin concentrations in the patient group were significantly lower ($P < 0·05$) than those observed in the healthy subjects.

The simple linear regression analysis of all samples (n = 45) from the three groups of subjects showed that there was a good correlation between the binding ratio of quinine and plasma AAG concentration ($r = 0·846$, $P < 0·005$; Fig. 1a). Significant correlations between the binding ratio and the plasma AAG concentration were also found in the group of malaria patients ($r = 0·813$, $P < 0·01$, n = 20), and healthy Thai subjects ($r = 0·679$, $P < 0·05$, n = 15). There was a weak linear relationship between the binding ratio of quinine and plasma albumin concentration but this was not significant even when all the data were combined ($r = 0·183$, $P > 0·05$, n = 45; Fig. 1b). This correlation coefficient was much less than that found in the case of the plasma AAG concentration.

Displacement of warfarin (site I) and diazepam (site II) by quinine

The effects of quinine on the binding of site I drug (warfarin) and site II drug (diazepam) were studied by adding quinine to the control plasma (580 μM albumin) up to a quinine concentration of 600 μM (195 μg mL^{-1}). Results showed that the percentage of unbound ligands (warfarin and diazepam) was not significantly changed over the quinine concentration range of 50–600 μM. Similar results were also observed when pure HSA solution (100 μM) was used and quinine was added at a concentration equimolar with albumin. In addition, increasing concentrations of site I drugs (warfarin and phenylbutazone) and site II drug (ibuprofen) up to 600 μM had no effect on quinine binding to normal plasma.

Fluorescent probe studies of quinine

Fig. 2 shows the effects of quinine on the fluorescence of

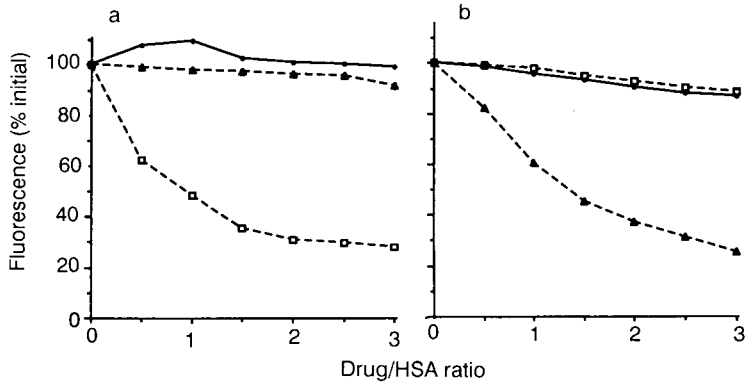

FIG. 2. Effects of quinine (●), phenylbutazone (□) and ibuprofen (△) on the fluorescence of probes selective for (a) site I (DNSA) or (b) site II (dansylsarcosine). Essentially fatty acid-free HSA was used at a concentration of 20 μM in 0·1 M sodium phosphate buffer containing 0·9% NaCl (pH 7·4). DNSA and dansylsarcosine concentrations were 2 μM. Fluorescence was measured at 475 nm with excitation at 350 nm. Fluorescence is expressed as a percentage of that before addition of displacing drugs.

Table 2. The effect of various drugs on the binding of quinine to normal human plasma proteins.

Drug	Concn (μg mL^{-1})	Unbound quinine (% control)
Acidic drugs		
Diclofenac	2	89·8
Ketoprofen (site II)	5	101·7
Naproxen (site II)	40	104·9
Phenylbutazone (site I)	100	89·2
Phenytoin	20	92·8
Salicylic acid	300	103·1
Warfarin (site I)	2	101·5
Basic drugs		
Alprenolol	0·2	95·8
Diazepam (site II)	2	95·5
Indapamide	0·25	90·6
Labetalol	0·25	94·1
Lignocaine	5	100·7
Pindolol	0·05	92·3
Propranolol	1	96·9
Antimalarial drugs		
Chloroquine	0·2	99·9
Mefloquine	0·5	90·7
Primaquine	0·2	89·2
Proguanil	0·5	89·0
Pyrimethamine	0·4	92·8

Pooled drug-free plasma from normal volunteers was used. Quinine concentration was 3 μg mL^{-1}. The percentage of unbound quinine in the absence of other drugs was 21·9. Each value represents the mean of duplicate analyses.

DNSA (site I probe) and dansylsarcosine (site II probe). Effects of the site I drug phenylbutazone and the site II drug ibuprofen (Sudlow et al 1975; Sjoholm et al 1979; Wanwimolruk et al 1983) are shown for comparison. Quinine caused no change in the fluorescence of either DNSA or dansylsarcosine. This suggests that quinine does not displace these specific fluorescent probes from their binding sites. As expected, phenylbutazone (site I drug) displaced DNSA, the site I probe, but not dansylsarcosine, whereas ibuprofen (site II drug) only displaced dansylsarcosine.

Effects of antimalarial drugs and other drugs on quinine binding

A number of acidic and basic drugs were tested for effects on the binding of quinine (Table 2) including commonly used antimalarial drugs. These drugs were added at therapeutic concentrations to control plasma. No marked displacement was observed with the drugs tested. This may be because these drugs are present in therapeutic concentrations well below quinine concentration (3 μg mL^{-1}). The plasma sample used contained 580 μM albumin and 15 μM AAG (0·6 g L^{-1}). When lignocaine was added to plasma at a concentration of 100 μM, the percentage unbound quinine was 34 ± 2% as compared with a control value of 21 ± 2% ($P < 0·05$, n = 4). On the other hand, an acidic drug, salicylic acid, did not cause any significant change in quinine binding when salicylate was added to control plasma up to 2000 μM (276 μg mL^{-1}).

Discussion

For drugs with a basic character, binding to AAG is generally more extensive than that to plasma albumin (Kremer et al 1988). Our data on quinine binding in the pure protein solutions of HSA and AAG support this view. The concentration of albumin was 40 g L^{-1} (600 μM) which was approximately 30-fold greater than the concentration of AAG used (0·7 g L^{-1} or 17·5 μM). Despite this, the percentage of unbound quinine in HSA solution was similar to that observed in the AAG solution (Table 1). Quinine was 35 and 34% bound to the protein in HSA and AAG solutions, respectively. The degree of quinine binding in normal plasma was found to vary from 69 to 92% in 25 plasma samples from healthy volunteers. These results confirm the previous studies (Mihaly et al 1987) that albumin and AAG are the major binding proteins in human plasma for quinine binding. The difference in percent binding in plasma and the sum of percent binding to albumin and AAG might be due to some other proteins such as lipoproteins in plasma. As there was a similarity in the extent of quinine binding between the AAG and the HSA solutions, even though the concentration of AAG used was much lower than albumin, this suggests that quinine was more avidly bound to AAG than to albumin. This is in accordance with the previous study (Mihaly et al 1987), although they used bovine serum albumin instead of HSA.

Our results showed drug concentration-dependent binding of quinine over the concentration range that would be expected during therapy (0–10 $\mu g\ mL^{-1}$). The results are in agreement with those reported by Mihaly et al (1987). However, other investigators did not find any dependency of quinine binding on the total drug concentration (Silamut et al 1985; Mansor et al 1991). Other basic drugs such as quinidine, lignocaine and disopyramide are highly bound to AAG and show non-linear pharmacokinetics due to binding dependency (Fremstad et al 1976; Meffin et al 1979; Routledge et al 1980; Edwards et al 1984).

Inter-racial differences in plasma protein binding of drugs between caucasians and oriental subjects have previously been reported with diphenhydramine, disopyramide and propranolol (Spector et al 1980; Zhou et al 1989, 1990). The higher binding of these basic drugs in caucasians was found to be associated with a higher plasma concentration of the acute-phase reactant AAG in these subjects (Zhou et al 1990). The possibility of inter-racial difference in the binding of quinine was examined in caucasians and Thai subjects. The ethnic origin of Thai people is relatively close to the Chinese. The present study has shown a tendency toward the direction of quinine binding being higher in caucasians but the difference in the unbound fractions was not significant (Table 1). The healthy subjects of Thai descent had a significantly lower concentration of AAG, compared with the caucasian subjects (Table 1). This is similar to that reported with Chinese subjects vs caucasians in the study by Zhou et al (1990). The failure to show a significant difference in the quinine binding between caucasian and Thai subjects may be due to the small number of subjects or contribution of other plasma proteins to quinine binding. The latter is supported by our finding that only approximately 70% of the variability in quinine binding in plasma samples (from both healthy subjects and patients with malaria) was accounted for by the variability in plasma AAG concentration ($r = 0{\cdot}846$, Fig. 1a).

In agreement with the previous studies (Silamut et al 1985, 1991; Mansor et al 1991), the binding of quinine in plasma obtained from patients with malaria was substantially higher than that in normal Thai subjects (Table 1). The higher binding of quinine in the patients with malaria is consistent with the higher plasma AAG concentration. The significant correlation ($r = 0{\cdot}846$) between the binding ratio of quinine and plasma AAG concentration (Fig. 1a) suggests that AAG is the major determinant of variability in protein binding of quinine. Our findings are in agreement with those reported previously (Mansor et al 1991; Silamut et al 1991). While plasma AAG concentration in patients with malaria is elevated, there is a compensatory decrease in plasma albumin concentration (Silamut et al 1985; Mansor et al 1990). There was no significant correlation between the binding ratio of quinine and plasma albumin concentration ($r = 0{\cdot}183$, $P > 0{\cdot}05$). A similar observation has been reported recently in a study carried out in Thai patients with acute falciparum malaria (Silamut et al 1991). This indicates that plasma albumin concentration is not a major determinant of variability in the binding of quinine.

Selective binding sites have been described for drugs on HSA. Specific drug binding sites I and II are mainly, but not exclusively, relevant to acidic drugs and some basic drugs, providing the basic drugs are un-ionized at plasma pH (Sudlow et al 1975; Sjoholm et al 1979; Fehske et al 1981; Wanwimolruk et al 1982, 1983; Birkett & Wanwimolruk 1986). Site I, also called the warfarin binding site, binds bulky heterocyclic molecules with enol group(s) carrying a negative charge in the centre of the molecule (Sudlow et al 1975; Birkett & Wanwimolruk 1986). Examples of drugs bound to site I are warfarin and phenylbutazone. Site II, which has also been called the indole and benzodiazepine binding site, binds arylpropionic acid non-steroidal anti-inflammatory drugs, including ibuprofen, flurbiprofen and naproxen, as well as some basic drugs such as diazepam (Sjoholm et al 1979; Wanwimolruk et al 1983). Both equilibrium dialysis and fluorescent probe displacement studies show that quinine does not bind to either of these two specific binding sites on HSA. This indicates that albumin acts as a low-affinity, high capacity binding protein for quinine in plasma.

Displacement of quinine by other drugs including the highly protein-bound antimalarial drugs, chloroquine, mefloquine and pyrimethamine, was tested with these drugs added at their plasma therapeutic concentrations (Table 2). Under these conditions, no marked displacement occurred.

In summary, the results presented in this report show that quinine binds to both albumin and AAG. The significant relationship between plasma AAG concentration and the binding of quinine in plasma, strongly suggests that this protein is the major determinant of variability in plasma protein binding of quinine. Plasma protein binding of quinine was greater in the patients with falciparum malaria, compared with the healthy subjects. This change in binding was attributable to the increase in plasma AAG concentration observed in the patients with malaria. The increase in plasma protein binding of quinine during malaria attack may have pharmacokinetic and toxicological consequences. Quinine was shown to be a drug with low to intermediate clearance (Shann et al 1985), and hence changes in protein binding will not affect the free (unbound) drug concentration at steady-state (Wilkinson & Shand 1975). This consequence may explain the previous observations by White et al (1982) that serious quinine toxicity is unusual in severe malaria patients, despite high plasma drug concentrations. With the proviso that good correlations exist between plasma AAG concentration and quinine binding, we suggest that plasma AAG may serve as a useful index to predict changes in quinine binding.

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References

Birkett, D. J., Wanwimolruk, S. (1986) Albumin as a specific binding protein for drugs and endogenous compounds. In: Tillement, J.-P., Lindenlaub, E. (eds) 20th Symposia Medica Hoechst: Protein Binding and Drug Transport. FK Schattauer Verlag, Stuttgart, pp 11–27

Duncombe, W. G. (1964) The colorimetric micro-determination of non-esterified fatty acids in plasma. Clin. Chim. Acta 9: 122–125

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

Fremstad, D., Bergerud, K., Haffner, J. F. W., Lunde, P. K. M. (1976) Increased plasma binding of quinidine after surgery: a preliminary report. Eur. J. Clin. Pharmacol. 10: 441–444

Fehske, K. J., Muller, W. E., Wollert, U. (1981) The location of drug binding sites in human serum albumin. Biochem. Pharmacol. 30: 687–692

Koch-Weser, J., Sellers, E. M. (1976) Binding of drugs to serum albumin. N. Engl. J. Med. 294: 526–531

Kremer, J. M. H., Wilting, J., Janssen, L. H. H. (1988) Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol. Rev. 40: 1–47

Mansor, S. M., Molyneux, M. E., Taylor, T. E., Ward, S. A., Wirima, J. J., Edwards, G. (1991) Effect of *Plasmodium falciparum* malaria infection on the plasma concentration of α_1-acid glycoprotein and the binding of quinine in Malawian children. Br. J. Clin. Pharmacol. 32: 317–321

Mansor, S. M., Taylor, T. E., McGrath, C. S., Edwards, G., Ward, S. A., Wirima, J. J., Molyneux, M. E. (1990) The safety and kinetics of intramuscular quinine in Malawian children with moderately severe falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 84: 482–487

Meffin, P. J., Robert, E. W., Winkle, R. A., Harapat, S., Peters, F., Harrison, D. C. (1979) Role of concentration-dependent plasma protein binding in disopyramide disposition. J. Pharmacokinet. Biopharm. 7: 29–46

Mihaly, G. W., Ching, M. S., Klejn, M. B., Paull, J., Smallwood, R. A. (1987) Differences in the binding of quinine and quinidine to plasma proteins. Br. J. Clin. Pharmacol. 24: 769–774

Piafsky, K. M. (1980) Disease-induced changes in the plasma binding of basic drugs. Clin. Pharmacokinet. 5: 246–262

Routledge, P. A. (1986) The plasma protein binding of basic drugs. Br. J. Clin. Pharmacol. 22: 499–506

Routledge, P. A., Barschowsky, A., Bjornsson, T. D., Kitchell, B. B., Shand, D. G. (1980) Lidocaine plasma protein binding. Clin. Pharmacol. Ther. 27: 347–351

Shann, F., Stace, J., Edstein, M. (1985) Pharmacokinetics of quinine in children. J. Paediatrics 106: 506–510

Silamut, K., Molunto, P., Ho, M., Davis, T. M. E., White, N. J. (1991) α_1-Acid glycoprotein (orosomucoid) and plasma protein binding of quinine in falciparum malaria. Br. J. Clin. Pharmacol. 32: 311–315

Silamut, K., White, N. J., Looareesuwan, S., Warrell, D. A. (1985) Binding of quinine to plasma proteins in falciparum malaria. Am. J. Trop. Med. Hyg. 24: 681–686

Sjoholm, I., Ekman, B., Kober, A., Ljungstedt-Pahlman, I., Seiuing, B., Sjodin, T. (1979) Binding of drugs to human serum albumin. Mol. Pharmacol. 16: 767–777

Spector, R., Choudhury, A. K., Chiang, C. K., Goldberg, M. J., Ghoneim, M. M. (1980) Diphenhydramine in orientals and caucasians. Clin. Pharmacol. Ther. 28: 229–234

Sudlow, G., Birkett, D. J., Wade, D. N. (1975) The characterization of two specific binding sites on human serum albumin. Mol. Pharmacol. 16: 824–832

Vallner, J. J. (1977) Binding of drugs by albumin and plasma protein. J. Pharm. Sci. 66: 447–465

Wanwimolruk, S., Birkett, D. J., Brooks, P. M. (1982) Protein binding of GP53,633: basic non-steroidal anti-inflammatory drug. Biochem. Pharmacol. 31: 3737–3743

Wanwimolruk, S., Birkett, D. J., Brooks, P. M. (1983) Structural requirements for drug binding site II on human serum albumin. Mol. Pharmacol. 24: 458–463

White, N. J. (1985) Clinical pharmacokinetics of anti-malarial drugs. Clin. Pharmacokinet. 10: 187–215

White, N. J., Looareesuwan, S., Warrel, D. A., Warrel, M. J., Bunang, D., Harinasuta, T. (1982) Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria. Am. J. Med. 73: 564–572

Wilkinson, G. R., Shand, D. G. (1975) A physiological approach to hepatic drug clearance. Clin. Pharmacol. Ther. 18: 377–390

Zhou, H., Adedoyin, A., Wilkinson, G. R. (1990) Differences in plasma binding of drugs between caucasians and Chinese subjects. Ibid. 48: 10–17

Zhou, H. H., Koshakji, R. P., Silberstein, D. J., Wilkinson, G. R., Wood, A. J. J. (1989) Racial differences in drug responses: altered sensitivity to and clearance of propranolol in men of Chinese descent as compared to American whites. N. Engl. J. Med. 320: 565–570

Zoest, A. R., Wanwimolruk, S., Hung, C. T. (1990) Simple high-performance liquid chromatographic method for the analysis of quinine in human plasma without extraction. J. Liq. Chromatogr. 13: 3481–3491