Br J clin Pharmac 1995; **39**: 519–526

The antimalarial drug halofantrine is bound mainly to low and high density lipoproteins in human serum

BRUNO CENNI[1]*, JOSEPH MEYER[2], ROGER BRANDT[2] & BRUNO BETSCHART[1,†]
[1]Department of Medical Parasitology, Swiss Tropical Institute, CH-4002 Basel and [2]Department of Pharmacokinetics, F. Hoffmann-La Roche, CH-4002 Basel, Switzerland

1 The major serum proteins which bind halofantrine were identified by size exclusion chromatography. In addition, the binding affinity of halofantrine to human erythrocytes and serum proteins was measured by an erythrocyte partitioning technique. The influence of serum-drug binding on the distribution of halofantrine in whole blood was estimated by simulating several disease-related changes in the levels of the most important binding proteins.

2 The chromatographic resolution of serum preincubated with halofantrine allowed a quantitative analysis of binding to low density lipoproteins, high density lipoproteins, α_1-acid glycoprotein and albumin using the erythrocyte partitioning technique. Very low density lipoproteins did not bind halofantrine to a significant extent.

3 In whole blood halofantrine is bound to serum proteins (83%) and to erythrocytes (17%). Low density lipoproteins (affinity constant nK_P = 44.4 l g^{-1}) and high density lipoproteins (nK_P = 14.4 l g^{-1}) were the most important binding proteins in serum. α_1-acid glycoprotein (nK_P = 4.39 l g^{-1}) and albumin (nK_P = 0.27 l g^{-1}) had relatively low binding affinities.

4 The concentration of serum proteins influences both the fraction of unbound drug and the fraction of drug associated with the erythrocytes. Changes in serum protein concentrations often encountered in malaria are likely to increase both the unbound fraction and the fraction bound to the erythrocytes.

Keywords halofantrine *Plasmodium falciparum* malaria pharmacokinetics serum protein binding

Introduction

Halofantrine was introduced for the treatment of chloroquine-resistant malaria [1]. The drug is generally well tolerated by adults and children and is highly active against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*. Recent reports of cardiac side effects emphasise the importance of a better understanding of factors that influence the distribution of halofantrine and which could modulate its pharmacological and toxic effects [2–4].

It is generally accepted that the pharmacological and toxic effects of drugs often correlate better with free (= plasma water) drug concentration than with the total plasma or blood drug concentration [5]. Halofantrine is thought to bind extensively to plasma proteins, but quantitative information is lacking.

Since halofantrine is a highly lipophilic drug that avidly adsorbs to various materials, methods to determine drug-protein binding, such as equilibrium dialysis, ultrafiltration or ultracentrifugation are difficult to apply [6]. Consequently, an alternative method as described by Urien and coworkers [7–11] was used in the present study. This method uses a dialysis system, in which protein solutions and erythrocytes are the two dialysis compartments, separated by the erythro-

Correspondence: Dr B. Betschart, Institut de Zoologie, Université de Neuchatel, Rue Emile-Argand 11, CH-2007 Neuchatel, Switzerland
*Present address: Cancer Center and Department of Medicine 0812, University of California San Diego, La Jolla, CA 92093-0812, USA
†Present address: Institut de Zoologie, Université de Neuchatel, CH-2007 Neuchatel, Switzerland

© 1995 Blackwell Science Ltd

cyte membrane. Since this method avoids the measurement of low concentrations of free drug in aqueous solutions, the assessment of the protein binding of highly lipophilic and adsorbing drugs is possible.

The purpose of the present study was to identify the serum proteins involved in the binding of halofantrine and to estimate their contribution to serum and blood binding under normal and pathological conditions.

Methods

Chemicals

All chemicals were of analytical grade. Halofantrine is (±)-1,3-dichloro-α-[2-(dibutylamino)ethyl]-6-(trifluoromethyl)-9-phenanthrenemethanol. Halofantrine hydrochloride and ring-labelled halofantrine hydrochloride ([^{14}C]-halofantrine hydrochloride) were kindly supplied by SmithKline Beecham (Welwyn Garden City, UK). The radiolabelled drug was purified by h.p.l.c. using a Varian Micropak SP-C18 reverse-phase column (4.0 mm i.d./15 cm length) and a Kontron isocratic pump system. Elution conditions were as described by Mberu *et al.* [12]. The final specific activity was 31.75 μCi mg^{-1} and the radiochemical purity was ≥97%, as measured by high performance thin layer chromatography (aluminium sheets precoated with silica gel G60 for nano t.l.c. from Merck; Darmstadt, Germany) using the elution system toluene:methanol:ammonia = 90:10:0.001 v/v. A stock solution of [^{14}C]-halofantrine in methanol (3.75 μCi ml^{-1}) was stored at 4° C until used. No significant change in radiochemical purity was observed over the experimental period.

Blood fractions

Blood was collected fresh from healthy volunteers into Vacutainer® tubes containing EDTA-K_3 (Becton Dickinson; Meylan, France). Plasma was separated from erythrocytes by centrifugation at 2800 rev min^{-1} for 10 min in a Sorvall RC3 centrifuge. The erythrocytes were washed three times with NaCl (0.9 g l^{-1}) and the buffy coat was discarded. Normal human serum (NHS) was prepared from fresh blood in SST Gel and Clot Activator Vacutainer® tubes (Becton/Dickinson; Meylan, France) and used individually or pooled. Human serum albumin (HSA, fatty acid free; A-1887) and α_1-acid glycoprotein (AAG; G-9885) were from Sigma (Buchs, Switzerland). High density lipoprotein (HDL, density range 1.063–1.21 g ml^{-1}) and low density lipoprotein (LDL, density range 1.019–1.063 g ml^{-1}), both isolated from fresh human plasma by sequential isopycnic ultracentrifugation, were from Cappel Research Products (Durham, USA).

Size exclusion chromatography

Normal human serum (NHS) was spiked with [^{14}C]-halofantrine (final concentration 4.7 ng μl^{-1}) and incubated at 20° C for 1 h. Aliquots of 100 μl were chromatographed at room temperature on a Fast Protein Liquid Chromatography (f.p.l.c.) system (Pharmacia, Uppsala, Sweden). A Superose 6 HR 10/30 column (separation range: 5000–5 × 10^6, Pharmacia, Sweden) was used with an elution buffer consisting of 20 mM HEPES pH 7.4, 5 mM EDTA and 150 mM NaCl. The maximal amount of serum that could be injected was 100 μl. The samples were eluted at a flow rate of 0.4 ml min^{-1} and fractions of 500 μl were collected over 75 min. The protein concentration was monitored by measuring the absorbance of the eluent at 280 nm. The chromatography system was calibrated for molecular mass estimation. The fractions were collected and either assayed for [^{14}C]-halofantrine in 5 ml Ecoscint A (National Diagnostics, USA) scintillation counting cocktail in a Kontron MR 300 (Kontron, Switzerland) β-counter, or used for protein characterisation as described below.

SDS-PAGE and Western blot analysis

SDS-PAGE was carried out in a discontinuous system with a 3% stacking gel and a 5–15% running gel [13]. The proteins were separated under non-reducing conditions at 10° C with a constant current of 20 mAmp for the first hour and 30 mAmp over the following 4 h. The running buffer was 50 mM TRIS, 385 mM glycine and 0.1% w/v SDS. The proteins in the fractions collected from the size exclusion chromatography were precipitated with an ice cold solution of 80% v/v acetone in water. The protein pellets were washed twice in ice cold acetone and redissolved in sample buffer to obtain equivalent protein concentrations. The proteins separated by SDS-PAGE were either stained with Coomassie Brilliant Blue R-250 or transferred electrophoretically onto a nitrocellulose sheet for immunological detection using specific antibodies [14]. Phosphate-buffer saline (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4 and 1.5 mM KH_2PO_4, pH 7.2) containing 5% w/v milk powder was used as blocking agent. The markers for HDL and LDL (apolipoprotein AI and B, respectively) were detected after reaction with monoclonal antibodies and colour development using a commercial alkaline phosphatase/BCIP/NBT kit (Immun-Blot; Bio-Rad, USA). The antibodies were generous gifts from Dr R. W. James (antiApoAI; University Hospital, Geneva, Switzerland) and Dr B. Schlapfer (antiApoB; The Swiss Red Cross, Berne, Switzerland).

Erythrocyte partitioning method

The erythrocyte partitioning method assumes that the free drug concentration in plasma is in equilibrium with the drug associated with the erythrocytes. To minimise losses due to non-specific drug adsorption, all glassware was siliconised by treatment with Sigmacote® (Sigma; Buchs, Switzerland). Washed human erythrocytes were resuspended in buffer (20 mM TRIS, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgSO_4$ and 5 mM glucose; pH 7.4) containing increasing concentrations of isolated proteins or of

NHS. The haematocrit was set to 0.2 with the total volume being 2.0 ml. The erythrocyte suspensions were spiked with a constant amount of [^{14}C]-halofantrine (5 μl of a methanolic solution), yielding a final drug concentration of 0.59 μM (= 41 000 d min^{-1}). All incubations were performed at 37° C under continuous shaking for 30 min.

At the end of the incubation the haematocrit (H) was measured in each sample and [^{14}C]-halofantrine concentration measured in duplicate in the whole suspension (C_{tot}) and supernatant (C_P) by liquid scintillation counting in a LS 6000LL β-counter (Beckman, USA). Counting efficiency was in the range of 70–80% for all samples. Drug concentration in the whole suspension (C_{tot}) was determined after digestion of 50 μl aliquots with 200 μl of a 1:1 mixture of Isopropanol/Soluene 350 (Packard, USA), and bleaching with 200 μl H_2O_2 and addition of 18 ml Ultima Gold scintillation liquid (Packard, USA). The drug concentration in the supernatant (C_P) was measured after separation of the supernatant from erythrocytes by centrifugation at 2500 rev min^{-1} for 5 min in a Heraeus Medifuge. Aliquots of supernatant (100 μl) were counted directly in 5 ml Ultima Gold scintillation liquid. In order to minimise temperature dependent variations the whole experiment was performed in a hood maintained at a temperature of 37° C until the final separation step. Each tube was rinsed twice with cold suspension buffer to remove protein- and erythrocyte-bound halofantrine. The drug adsorbed to the tube wall was collected by washing with 4 ml methanol. The radioactivity in these samples was determined as described for the supernatants.

Calculations

The concentration ratio C_E/C_P defines drug distribution between erythrocyte and supernatant and is calculated from the following equation:

$$C_E/C_P = \frac{(C_{tot}/C_P) - (1 - H)}{H} \quad (1)$$

where H is the haematocrit and C_{tot}, C_E and C_P denote the total drug concentrations in the whole suspension, erythrocytes and protein solution, respectively. Using halofantrine concentrations of 0.59 μM, which are orders of magnitude lower than nK_P, the total and free drug concentrations in the protein solution and in the erythrocytes were linearly related as follows [7–11]:

$$C_P = (1 + nK_P \cdot P_t) \cdot C_u \quad (2)$$

$$C_E = (1 + NK_E) \cdot C_u \quad (3)$$

where P_t is the total concentration of protein, C_u is the concentration of free drug, nK_P is the total binding constant for each drug-binding interaction (product of n, the number of binding sites, and K_P, the association constant for each protein). N is then the concentration of binding sites ($N = n \cdot P_t$; product of n, number of binding sites, and P_t, the receptor or protein concentration). Thus, NK_E is the binding capacity of the erythrocyte (dimensionless, defined as the concentration ratio between erythrocyte-bound and free drug). In this model no distinction between intraerythrocytic and erythrocyte bound drug is necessary. Dividing equation (2) by equation (3) yields:

$$C_P/C_E = \frac{(1 + nK_P \cdot P_t)}{(1 + NK_E)} \quad (4)$$

If only serum binding is considered, equation (2) is modified to:

$$C_b/C_P = \frac{(nK_P \cdot P_t)}{(nK_P \cdot P_t) + 1} = \frac{NK_P}{(NK_P + 1)} \quad (5)$$

where C_b is the concentration of bound drug.

Thus, the ratio C_P/C_E is linearly related to the protein concentration (P_t) in the solution. The C_P/C_E ratios were measured for several proteins and a series of concentrations. The data were analysed according to equation (4) by an iterative non-linear regression program using the least-squares criterion (Micropharm, INSERM 1993).

Simulations of blood distribution

Theoretical calculations were carried out using the same software to simulate the fraction of halofantrine bound to erythrocytes and to each protein fraction in blood, as well as the amount of free drug, as described by Urien *et al.* [7] and Albengrès *et al.* [15]. Serum protein concentrations were chosen according to Graninger *et al.* [16], Mohanty *et al.* [17] and Braunwald *et al.* [18].

Results

Halofantrine-binding to serum proteins

In order to restrict quantitative analysis of binding parameters to the main drug binding proteins and to estimate their relative contribution to overall binding, we identified halofantrine-binding serum proteins by size-exclusion chromatography. To obtain a measurable number of counts in the eluent, a high concentration of labelled drug (470 ng 100 μl^{-1}) had to be used. The recovery of radioactivity in the eluent did not exceed 40% of the injected amount, owing to extensive non-specific adsorption of halofantrine to the chromatographic system. For the same reason it was not possible to produce an elution profile for [^{14}C]-halofantrine alone, as no radioactivity could be recovered without an additional washing step requiring an injection of unlabelled serum proteins. The elution profiles of serum proteins were reproducible and unaffected by the addition of methanolic drug solution. The vari-

ation between individual serum samples and pooled serum was negligible.

As shown in Figure 1, the elution profile of [^{14}C]-halofantrine indicated two major peaks of radioactivity in the fractions from 10.5–14.5 ml (peak 1) and in the fractions from 15.0–19.5 ml eluent volume (peak 2). The proteins in these fractions had a molecular mass in the range of 1000–450 kDa and 400–10 kDa, respectively. The serum proteins in this range generally known to bind drugs are human serum albumin (HSA, M_r 66 500), α_1-acid glycoprotein (AAG, M_r 44 000), high-density lipoproteins (HDL, M_r 0.3×10^6) and low-density lipoproteins (LDL, M_r 2.5×10^6). Lipoproteins of very low density (VLDL) have an apparent molecular weight of $3–130 \times 10^6$. Because the upper separation range limit of the column was 5×10^6, the VLDL eluted in the void volume of 7.7 ml. Since no radioactivity above background was detected in the region of the void volume, VLDL were not considered in further binding experiments. The presence of HDL/LDL and HSA/AAG was verified by subjecting the collected fractions to SDS-PAGE and Western blot analysis for the lipoproteins.

Figure 2 shows a Coomassie Blue stained gradient SDS-PAGE of proteins collected in the fractions from 7.5 ml to 22.5 ml eluent volume. The most prominent candidate binding proteins visible are HSA and AAG, mainly in lanes 7 and 8 at apparent molecular weights of 60 000 and 45 000 under non-reducing conditions. Figure 3 shows a Western blot of the SDS-PAGE lanes 3–8 developed with monoclonal antibodies apolipoprotein AI and B. HDL and LDL references were run in both blots in lanes R1 (HDL) and R2 (LDL), respectively. As expected, LDL is mainly present in lanes 3–5 and HDL mainly in lanes 5–7. Thus, in the serum protein elution profile LDL is mainly found in fractions from 10.5–15.0 ml and HDL in the fractions from 13.5–18.0 ml.

The [^{14}C]-halofantrine found in peak 1 of the size-exclusion chromatogram is attributed mainly to the drug bound to LDL, whereas peak 2 is attributed to drug associated with HDL, HSA or AAG.

Estimation of binding to serum proteins

Preliminary experiments showed that equilibrium distribution of halofantrine between suspended erythrocytes and protein solutions was achieved in less than 5 min and was stable for at least 2 h. The incubation time was therefore set at 30 min. The data obtained using the erythrocyte partitioning method were fitted by the theoretical model for all proteins. Figure 4 shows representative experimental data for halofantrine binding to diluted serum, HSA, AAG, HDL and LDL. The binding parameters obtained from these curves are summarised in Table 1. The binding affinity constants (nK_P) of the isolated proteins were 0.27 l g^{-1} for HSA, 4.39 l g^{-1} for AAG, 14.4 l g^{-1} for HDL and 44.4 l g^{-1} for LDL. In all experiments the goodness of fit could be improved by correction for the amount of drug adsorbed to the tube. Adsorption only occurred to a measurable extent at the lowest protein concentrations of each series and never exceeded 5% of the total drug concentration. The total binding capacity (NK_P) of NHS was 244. The sum of the binding capacities of the individual proteins, as calculated using physiological concentrations of each protein investigated, was slightly lower (216). Nevertheless, the sum of the corresponding serum binding

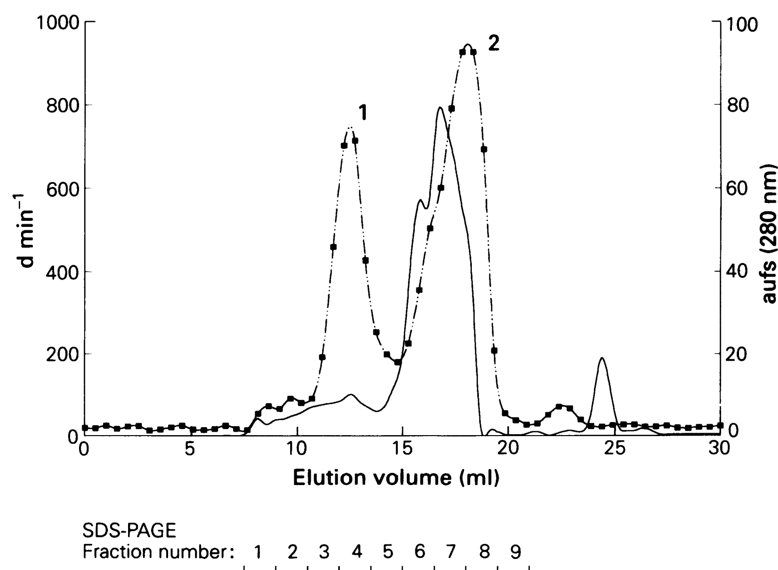

Figure 1 Resolution by SEC of serum preincubated with [^{14}C]-halofantrine. Column: Superose 6 HR 10/30 (separation range: 5000–5·10^6). Injection of 100 µl NHS preincubated with 470 ng [^{14}C]-halofantrine. Elution with buffer consisting of 20 mM HEPES pH 7.4, 5 mM EDTA, 150 mM NaCl and a flow rate of 0.4 ml min^{-1}. Solid line: absorbance at 280 nm, squares: d min^{-1} recovered in the eluent. The numbered peaks of radioactivity are discussed in the text. The bottom bar shows the fractions used for SDS-PAGE and Western blot analysis.

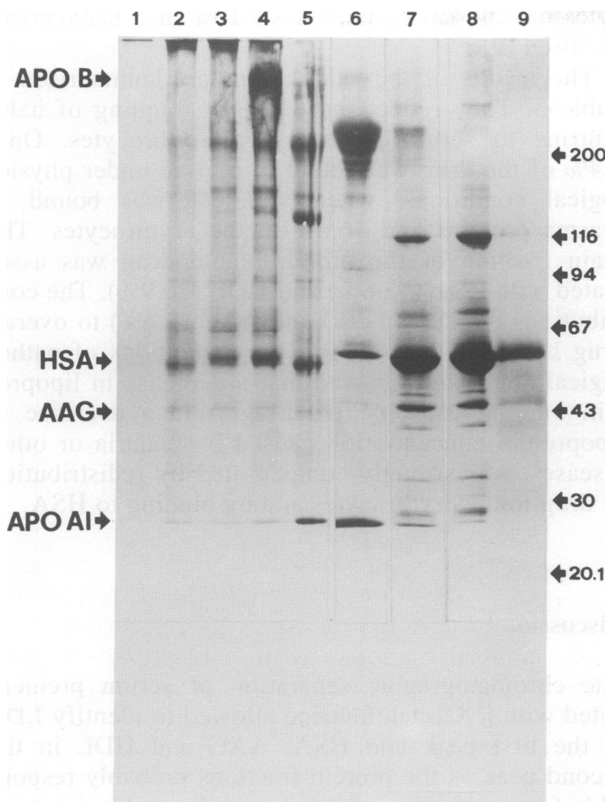

Figure 2 Non-reducing gradient SDS-PAGE of fractions collected from 7.5 ml to 21.0 ml eluent volume, stained with Coomassie Blue. The volume of each fraction was 1.5 ml. The lanes were numbered according to the elution sequence (e.g. fraction 1: eluent volume 7.5–9.0 ml in lane 1). In each lane 50 μl aliquots of equivalent protein concentration were loaded. Apparent molecular weights and running distances of isolated protein references are shown. The most prominent candidate drug binding proteins visible are HSA and AAG, mainly in lanes 7 and 8 at apparent molecular weights of 60 000 and 45 000.

percentages calculated according to equation (5) showed that binding to the chosen proteins accounted for more than 99.5% of the total serum protein binding.

Estimation of binding to erythrocytes

Binding to RBC was measured in several experiments both with isolated proteins and diluted serum. The mean value of the total erythrocyte binding capacity (NK_E = concentration ratio between erythrocyte bound and free drug) found in these experiments was 52 ± 14 (n = 5, s.d.). This is equivalent to an erythrocyte/serum concentration ratio of 0.2, and a blood/serum concentration ratio of 0.65, as calculated for a haematocrit of 0.45. This value was in good agreement with the blood/serum concentration ratio (C_{tot}/C_P) of 0.66 (H = 0.48) measured for [^{14}C]-halofantrine, and which was independent of total drug concentration up to 2500 ng ml^{-1}.

Mathematical simulation of blood distribution

Using the estimated binding parameters of halofantrine to the different blood fractions, the distribution of the drug in blood was simulated for physiological and several pathological conditions. The protein concentrations used in the simulations are shown in Table 2. Changes in protein levels were assumed according to patterns typically found in patients suffering from malaria, from extreme hypolipoproteinaemia to hyperlipoproteinaemia. To simulate the distribution changes associated with malaria, an erythrocyte binding capacity NK_E of 82 instead of 52 was used which reflected the accumulation of [^{14}C]-halofantrine found in *P. falciparum* parasitized erythrocytes *in vitro* incubation experiments in the presence of total serum with a population of erythro-

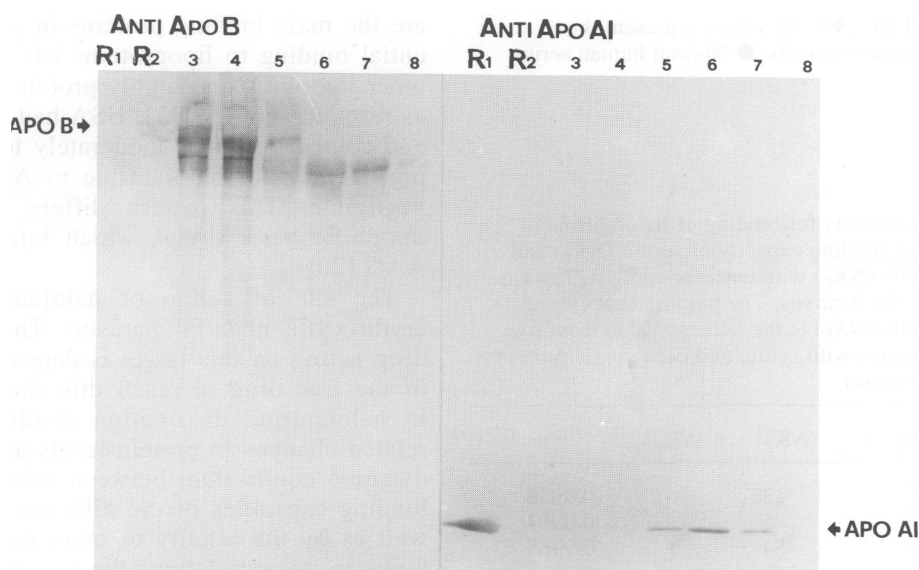

Figure 3 Western blot analysis of SDS-PAGE fractions 3–8 incubated with monoclonal antibodies against apolipoprotein AI and B. In both blots the fractions R1 are apolipoprotein AI containing HDL references and the fractions R2 apolipoprotein B containing LDL references. Apolipoprotein B is mainly present in lanes 3–7 and apolipoprotein AI mainly in lanes 5–7.

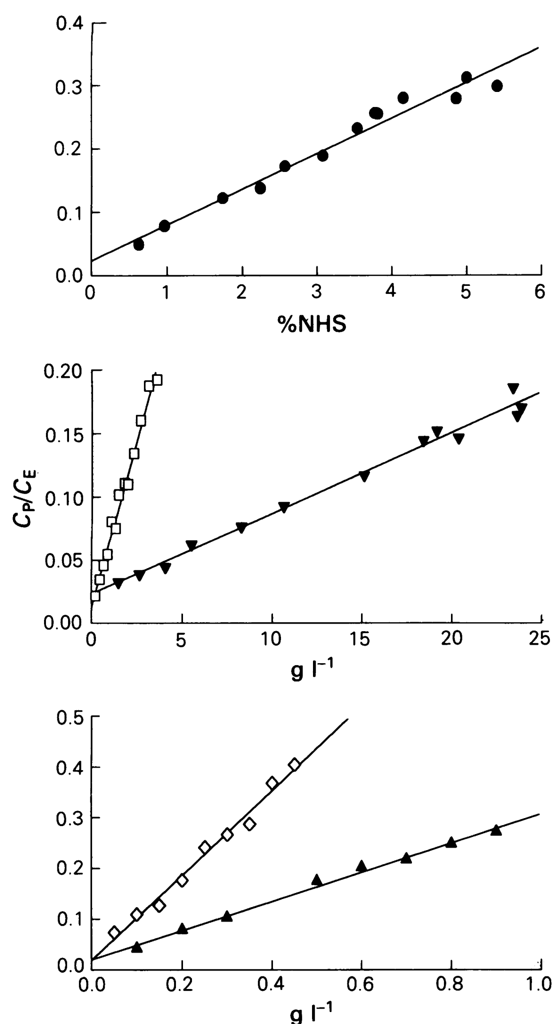

Figure 4 Partitioning of halofantrine between normal red blood cells and total serum, HSA, AAG, HDL and LDL. At equilibrium the ratio of the drug concentration in the protein-phase and in erythrocytes was determined and plotted against protein concentration. The lines represent linear fits (according to equation 4). X-axes are % dilution of total serum and g l^{-1} concentrations of HSA (▼), AAG (□), HDL (▲) and LDL (◆). All values represent the means of duplicate measurements. ● Normal human serum (NHS).

Table 1 Affinity constants for binding of halofantrine to single proteins (nK_P), binding capacity of serum (NK_P) and normal red blood cells (NK_E) with corresponding regression coefficients *r* of the fitted curves. The binding capacity of normal red blood cells (NK_E) is the average value from five independent experiments with serum and each single protein (s.d. = standard deviation)

Protein	nK_P ($l\ g^{-1}$)	NK_P[1]	NK_E[1]	r
HSA	0.27	13		0.9936
AAG	4.39	5		0.9913
HDL	14.4	54		0.9965
LDL	44.4	144		0.9921
NHS		244		0.9839
nRBC		52 ± 14 s.d.		

[1]Dimensionless.

cytes having a parasitaemia of 10% at a haematocrit of 50% [19].

The results of the calculations are summarised in Table 3. They reflect the extensive binding of halofantrine to serum proteins and erythrocytes. Only 0.4% of the drug was found to be free under physiological conditions, whereas 83.0% was bound to plasma proteins and 16.6% to the erythrocytes. The main fraction of the protein-bound drug was associated with LDL (55.8%) and HDL (20.9%). The contributions of HSA (4.7%) and AAG (1.6%) to overall drug binding were minor. The simulations of pathological conditions showed that a decrease in lipoprotein binding capacity, resulting from a decrease in lipoprotein concentration caused by malaria or other diseases, was strongly compensated by redistribution of drug to the erythrocytes and by binding to HSA.

Discussion

The chromatographic separation of serum preincubated with [^{14}C]-halofantrine allowed to identify LDL in the first peak and HSA, AAG and HDL in the second peak as the protein fractions probably responsible for the binding of this lipophilic and basic drug. The drug–protein interaction with serum proteins might be disturbed during chromatography by the adsorption of the drug to the column, since the elution conditions did not allow equilibrium between free and protein-bound drug to be reached. However, this effect would influence drug binding to proteins of low affinity, rather than to those of high affinity. Since it was possible to detect the drug bound with low affinity to AAG and HSA, we conclude that size-exclusion-chromatography is an adequate method to identify the major drug-binding serum proteins.

The binding data obtained from the erythrocyte partitioning experiments revealed that LDL and HDL, are the main binding proteins in serum. Such preferential binding to lipoproteins has been described for other lipophilic and highly protein-bound drugs, such as retinoid analogs [11]. HSA had a very low affinity and contributed only moderately to the overall binding. Binding of halofantrine to AAG, however, was negligible. This pattern differs from many other lipophilic basic drugs, which bind preferentially to AAG [20].

The site of action of halofantrine is the intra-erythrocytic malarial parasite. The concentration of drug acting on this target is dependent on the ability of the free drug to reach this site [7]. The changes in halofantrine distribution resulting from disease-related changes in protein-levels are determined by a dynamic equilibrium between unbound drug and the binding capacities of the different blood fractions as well as by the affinity to other compartments in the body. In the simulations the free fraction of the drug increased as the levels of the major binding proteins decreased. However, in whole blood this was compensated by an increase of the drug associated to erythrocytes. The calculations of the distribution of

Table 2 Serum protein concentrations used for simulations of halofantrine distribution in blood under physiological and pathological conditions

Protein	*Normal concentrations* ($g\ l^{-1}$)	*Malaria* ($g\ l^{-1}$)	*Abetalipo-proteinaemia* ($g\ l^{-1}$)	*Hyperlipo-proteinaemia* ($g\ l^{-1}$)
HSA	45.00	28.50	45.00	45.00
AAG	1.00	1.40	1.00	1.00
HDL	3.75	1.48	0.20	7.50
LDL	3.25	2.30	0.00	6.50

Table 3 Blood distribution of halofantrine. Results of calculations based on protein concentrations in healthy individuals and patients suffering from malaria, abetalipoproteinaema or hyperlipoproteinaemia. (Plasma = plasma-protein bound + free drug)

Protein	*Normal concentrations*	*Malaria*	*Abetalipo-proteinaemia*	*Hyperlipo-proteinaemia*
HSA	4.7	3.8	19.4	2.6
AAG	1.6	2.8	6.4	0.9
HDL	20.9	10.4	4.6	23.1
LDL	55.8	49.8	0.0	64.1
Free drug	0.4	0.5	1.6	0.2
Plasma	83.4	67.3	32.0	90.9
RBC	16.6	32.7	68.0	9.1

All values are expressed as % of the total drug associated to the individual protein fraction.

the drug in blood indicate that the total drug concentration in the erythrocytes is increased when the levels of LDL and HDL decrease. Such changes in lipoproteins are likely to be found in individuals suffering from malaria [16, 17]. On the other hand, in situations where there is an increase of binding by the lipoproteins, the level of total erythrocyte-associated drug will decrease. Such a situation may be encountered in individuals suffering from hyperlipoproteinaemic disease, which is frequent in industrialised countries and is increasing in urban populations in the tropics [21–23]. Since the effect on the parasite is not likely to be correlated with total parasite drug concentration, but rather with its free concentration and since its equilibration is probably not affected *in vivo*, changes in protein binding are not likely to affect the drug action on the parasite.

Toxic effects could be influenced by changes in lipoprotein levels, as the relative variation in free drug fraction is large for this highly bound drug. For example an increase of the unbound fraction from 0.2% calculated for hyperlipoproteinaemia to 1.6% for an extremely low lipoprotein level may seem small, but represents an eight-fold increase of the free fraction.

However, the *in vivo* situation might significantly differ from what is found *in vitro*, since drugs having a large apparent volume of distribution might compensate eventual changes in free fraction by redistribution of free drug into the body tissues. The volume of distribution of halofantrine has not yet been assessed reliably. Reports suggesting a large apparent volume of distribution (100–569 $l\ kg^{-1}$) are in contrast with others indicating lower values, probably resulting from the variable and low absorption after oral application compared with the only study published with parenteral application (3.1 $l\ kg^{-1}$ at steady state) (reviewed in [24]).

Halofantrine is extensively metabolised, but unlike other quinoline antimalarials it does not inhibit cytochrome P450-mediated drug metabolism [25, 26]. However, interactions between halofantrine and drugs that are able to displace it from its binding sites and drugs that inhibit its metabolism have not yet been investigated. Interactions with quinine which also shows cardiac side effects should be of concern in future studies. One singular case of such an interaction between halofantrine and quinine has been reported by Krishna *et al.* [2]. One patient in this study was given parenteral quinine (10 mg kg^{-1}) for treatment of a recrudescent parasitaemia after completion of the halofantrine regimen. This resulted in an impressive increase of the plasma halofantrine concentration to more than 4000 ng ml^{-1}. No clinical signs of increased cardiotoxicity were noticed and the analytical method used to assess halofantrine in plasma was not sensitive to the presence of quinine. Thus, the role of the displacement from binding sites in blood and tissue as well as the inhibition of drug metabolism should be further investigated.

Although halofantrine is extensively bound to proteins in serum the clinical significance of its protein binding remains to be determined in specific studies. According to an algorithm described by Rolan [27] the protein binding of halofantrine is not likely to be of clinical significance, unless halofantrine is given parenterally.

This work was financially supported by the Senglet Foundation, Basel (BC). We thank Jennifer Jenkins and Christian Burri for the critical reading of the manuscript and the many helpful discussions and Dr R. W. James and Dr B. Schlapfer for providing the specific antibodies. We also thank Dr P. Huguenin for his assistance in the purification of the labelled compound.

References

1 Horton RJ. Introduction of halofantrine for malaria treatment. *Parasitology Today* 1988; **4**: 238–239.

2 Krishna S, TerKuile FO, Supanaranond W, *et al.* Pharmacokinetics, efficacy and toxicity of parenteral halofantrine in uncomplicated malaria. *Br J clin Pharmac* 1993; **36**: 585–591.

3 Nosten F, TerKuile FO, Luxemburger C, *et al.* Cardiac effects of antimalarial treatment with halofantrine. *Lancet* 1993; **341**: 1054–1056.

4 Karbwang, J. Bangchang KN, Bunnag D, Harinasuta T, Laothavorn P. Cardiac effect of halofantrine. *Lancet* 1993; **342**: 501.

5 Tillement JP, Houin G, Zini R, *et al.* The binding of drugs to biological macromolecules in plasma. Recent advances and therapeutic significance. *Adv Drug Res* 1985; **13**: 59–94.

6 Pacifici GM, Viani A. Methods of determining plasma and tissue binding of drugs—pharmacokinetic consequences. *Clin Pharmacokin* 1992; **23**: 449–468.

7 Urien S, Riant P, Renouard A, Coulomb B, Rocher I, Tillement JP. Binding of indapamide to serum proteins and erythrocytes. *Biochem Pharmac* 1988; **37**: 2963–2966.

8 Maulard C, Urien S, Bastian G, Tillement JP. Binding of retelliptine, a new antitumoral agent, to serum proteins and erythrocytes. *Biochem Pharmac* 1990; **40**: 895–898.

9 Urien S, Zini R, Lemaire M, Tillement JP. Assessment of cyclosporine-A interactions with human plasma lipoproteins *in vitro* and *in vivo* in the rat. *J Pharmac exp Ther* 1990; **253**: 305–309.

10 Urien S, Bastian G, Lucas C, Bizzari JP, Tillement JP. Binding of a new vinca alkaloid derivative, A12363, to human plasma proteins and platelets. Usefulness of an erythrocyte partitioning technique. *Invest New Drugs* 1992; **10**: 263–268.

11 Urien S, Claudepierre P, Meyer J, Brandt R, Tillement JP. Comparative binding of etretinate and acitretin to plasma proteins and erythrocytes. *Biochem Pharmac* 1992; **44**: 1891–1893.

12 Mberu EK, Muhia DK, Watkins WM. Measurement of halofantrine and its major metabolite desbutylhalofantrine in plasma and blood by high-performance liquid chromatography—a new methodology. *J Chromatogr* 1992; **581**: 156–160.

13 Picconi R, Bellemare G, Chua NH. Methods of polyacrylamide gel electrophoresis in the analysis and preparation of plant polypeptides. In *Methods in chloroplast molecular biology*, ed. Edelmann, XX, pp. 985–1014. Elsevier, Amsterdam, 1982.

14 Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**: 4350–4354.

15 Albengrès E, Urien S, Pognat JF, Tillement JP. Multiple binding of bepridil in human blood. *Pharmacology* 1984; **28**: 139–149.

16 Graninger W, Thalhammer F, Hollenstein U, Zotter GM, Kremsner PG. Serum protein concentrations in *Plasmodium falciparum* malaria. *Acta Tropica* 1992; **52**: 121–128.

17 Mohanty S, Mishra SK, Das BS, *et al.* Altered plasma lipid pattern in *falciparum* malaria. *Ann Trop Med Parasitol* 1992; **86**: 601–606.

18 Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS. *Harrison's principles of internal medicine*, 12 Edition, p. 1814ff. McGraw-Hill, New York, 1991.

19 Cenni B, Betschart B. The *in vitro* distribution of halofantrine in human blood and *Plasmodium falciparum* parasitised red blood cells. *Chemotherapy* 1995; in press.

20 Müller WE, Stillbauer AE. Characterization of a common binding site of basic drugs on human α_1-acid glycoprotein. *Naunyn Schmiedeberg's Arch Pharmac* 1983; **322**: 170–173.

21 Beevers DG, Prince JS. Some recent advances in non communicable diseases in the tropics. 1. Hypertension: an emerging problem in tropical countries. *Trans R Soc Trop Med Hyg* 1991; **85**: 324–326.

22 Harpham T, Stephens C. Urbanization and health in developing countries. *WHO Statistics Quarterly* 1991; **44**: 62–69.

23 Korte R, Rehle T, Merkle A. Strategies to maintain health in the Third World. *Trop Med Parasitol* 1991; **42**: 428–432.

24 Karbwang J, Bangchang KN. Clinical pharmacokinetics of halofantrine. *Clin Pharmacokin* 1994; **27**: 104–119.

25 Milton KA, Hoaksey PE, Ward SA, Edwards G. Lack of effect of halofantrine on hepatic drug metabolism in the rat *in vivo* and *in vitro. Biochem Pharmac* 1990; **39**: 1581–1586.

26 Bangchang KN, Karbwang J, Back DJ. Primaquine metabolism by human liver microsomes: effect of other antimalarial drugs. *Biochem Pharmac* 1992; **44**: 587–590.

27 Rolan PE. Plasma protein binding displacement interactions—why are they still regarded as clinically important? *Br J clin Pharmac* 1984; **37**: 125–128.

(Received 27 July 1994, accepted 9 January 1995)