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# Effect of bile acids on the binding of drugs and dyes to human albumin

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Chenodeoxycholic, cholic, deoxycholic and taurodeoxycholic acids were found to inhibit the binding of 2-(4′-hydroxybenzeneazo)benzoic acid, methyl orange, sulphadimethoxine and warfarin to human albumin. In addition, glycodeoxycholic acid inhibited the binding of sulphadimethoxine and warfarin. In contrast, these bile acids did not inhibit the binding of phenylbutazone. The extent of displacement was in the rank order of: dihydroxy acids (chenodeoxycholic and deoxycholic) > trihydroxy acid (cholic) > conjugates (glycodeoxycholic and taurodeoxycholic). Thus the introduction of polar groups into the steroid nucleus of bile acids reduces their effectiveness as binding inhibitors. Displacement was usually accompanied by a decrease in the apparent association constant which suggests that the mechanism of displacement may be competitive.

Many weakly acidic drugs are known to bind to albumin and it is now well recognized that these ligands can displace each other from albumin binding sites. In some instances displacement can assume clinical importance especially if the increase in unbound drug results in a greater pharmacological effect. Albumin also binds many endogenous metabolites and these too can alter the albumin binding of drugs. The metabolites most investigated in this respect are bilirubin and non-esterified fatty acids (Vallner 1977); but comparatively little is known about the effect of other endogenous ligands such as bile acids on drug–albumin interactions.

Bile acids are known to bind to plasma proteins and in particular to albumin (Rudman & Kendall 1958; Burke et al 1971). Their interaction with albumin is extensive and seems to involve two classes of binding sites (Burke et al 1971; Roda et al 1982), the first class consisting of 2 to 3 sites whereas the second class contains some 8 to 30 sites (Roda et al 1982). The affinity of bile acids for these classes of binding sites range from $2 \times 10^5$ to $1{\cdot}8 \times 10^3$ M$^{-1}$ and $4 \times 10^4$ to $3 \times 10^2$ M$^{-1}$, respectively (Roda et al 1982) and decreases as polar groups are introduced into the steroid nucleus of the bile acid molecule (Rudman & Kendall 1958; Roda et al 1982).

Because of their extensive binding it seems possible that bile acids might share binding sites with other weakly acidic ligands and may, therefore, compete with these ligands for the limited number of binding sites on plasma albumin. Consequently, the aim of the present work was to assess the effect of a number of bile acids on the binding of various drugs and dyes to human albumin (HA).

* Correspondence.

*Materials and methods*

*Chemicals.* Crystalline human albumin (HA, fraction V, several lot numbers) was purchased from Sigma Chemical Co. Methyl orange and 2-(4′-hydroxybenzeneazo)benzoic acid (HABA) were obtained from BDH Ltd, and were recrystallized from water and methanol, respectively, before use. Phenylbutazone, sulphadimethoxine and warfarin were kindly donated by Geigy Pharmaceuticals, Roche Ltd and Ward Blenkinsop Ltd. All other reagents were available commercially and of analytical grade.

*Equilibrium dialysis.* Binding was measured at 37 °C by equilibrium dialysis using a 'Dianorm' apparatus (M.S.E. Fisons Ltd). Visking tubing (Medicell International) was used as the dialysis membrane and dialysis times were 2 h for the dyes and 3 h for the drugs using 1 ml half-cells. All experiments were done with 0·1 M sodium phosphate buffer, pH 7·4, and the recovery of each ligand from the dialysis cells, in the absence of HA, was >97%. The concentration of HA was 1% w/v (145 μM; assuming a mol. wt of 69 000) and the bile acid concentration in the half-cell containing HA before dialysis was 250 μM.

*Determination of unbound ligand.* The unbound concentrations of HABA, methyl orange, phenylbutazone and warfarin were measured spectrophotometrically at 348, 464, 263 and 308 nm, respectively. Sulphadimethoxine was determined by the method of Bratton & Marshall (1939).

*Analysis of results.* The results were analysed using Scatchard plots (Scatchard 1949) in which the molar ratio of bound ligand to albumin, r, is plotted against r/Du, where Du is the concentration of unbound ligand at equilibrium. Estimates of k, the apparent association constant, and n, number of binding sites, and their asymptotic standard deviations were obtained using derivative free non-linear regression analysis (Ralston 1983). The following equations were used:

$$r = nkDu - rkDu$$

for a straight line relationship and

$$r = \frac{n_1 k_1 Du}{1 + k_1 Du} + \frac{n_2 k_2 Du}{1 + k_2 Du}$$

for a curved relationship, where $n_1$ and $n_2$ represent the number of primary and secondary binding sites and $k_1$ and $k_2$ are the corresponding apparent association constants. The statistical significance of changes in k and n in the presence of bile acids was not assessed because the asymptotic standard deviations are only estimates whose precision is highly dependent upon both sample size and degree of correlation between estimated parameters (Ralston 1983).

Displacement was considered significant if the unbound concentration of ligand in the presence of bile acid was significantly greater ($P < 0.05$) than that in the absence of bile acid at the same total ligand concentration and if the regression lines on the Scatchard plots did not overlap with their respective control lines. To improve the clarity of the Scatchard plots, only experimental data for the control plot are shown, and binding in the presence of bile acids is represented by the relevant regression line. Each point is the mean of three or more experiments.

*Results*

*Dyes.* Fig. 1 depicts the Scatchard plots for HABA in the presence of 0 and 250 μM chenodeoxycholic, cholic, deoxycholic, glycodeoxycholic and taurodeoxycholic acids. Over the limited range of HABA concentrations used (20–100 μM), these plots were linear indicating that HABA occupied a single class of binding sites. All the bile acids, except glycodeoxycholic, decreased the binding of HABA and their effect upon the values of n and k is shown in Table 1. The principal effect of these acids was to decrease k and the extent of this decrease was in the order: dihydroxy acids (chenodeoxycholic and deoxycholic) > trihydroxy (cholic) > conjugates (glycodeoxycholic and taurodeoxycholic). The dihydroxy acids increased the unbound fraction by about 1·5-fold at total dye concentrations of 20 and 100 μM.

In contrast to HABA, methyl orange (30–300 μM) gave rise to a curved Scatchard plot suggesting the existence of more than one class of binding site for this dye. Analysis of these data using a two site model gave the values of $n_1$, $k_1$ and $n_2$, $k_2$ listed in Table 1.

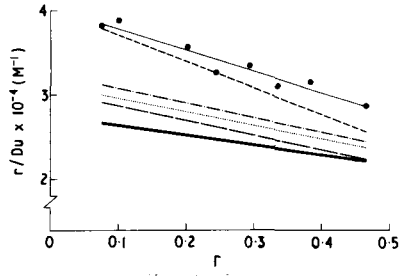


FIG. 1. Scatchard plot for the binding of HABA to 1% w/v HA in the absence (——) and presence of glycodeoxycholic acid (– – – –), taurodeoxycholic acid (— · — ·), cholic acid (· · · ·), chenodeoxycholic acid (— —) and deoxycholic acid (▬▬). Bile acid concentration was 250 μM.

Glycodeoxycholic acid was not tested because it failed to displace the less highly bound HABA; but all the other bile acids decreased methyl orange binding and the order of potency was similar to that observed with HABA. The main effect of bile acids appeared to be on the high affinity binding sites where $k_1$ was substantially reduced (Table 1). However, deoxycholic acid had a pronounced effect on both $k_1$ and $k_2$. The increase in unbound fraction in the presence of the dihydroxy acids was about 35 and 2-fold at 30 and 300 μM, respectively.

*Drugs.* The binding of phenylbutazone to HA was not altered by any of the bile acids tested. Over the range of concentrations used (80–322 μM) sulphadimethoxine gave a linear Scatchard plot (Fig. 2) and all the bile acids, including glycodeoxycholic, produced a significant displacement. As previously found with the dyes, the principal effect of the bile acids was to decrease the apparent association constant, k (Table 2). The dihydroxy acids (chenodeoxycholic and deoxycholic) were more potent inhibitors of binding than either the trihydroxy acid (cholic) or the conjugates (glycodeoxycholic and taurodeoxycholic) and they increased the unbound fraction of drug by 4 and 1·5-fold at 80 and 322 μM, respectively.

Scatchard plots for the binding of warfarin (83–1250 μM) in both the absence and presence of bile

Table 1. Effect of various bile acids on the apparent association constant (k) and number of binding sites (n) for HABA and methyl orange.

| | HABA | | Methyl orange | | | |
|---|---|---|---|---|---|---|
| Bile acid | n | k $(M^{-1}) \times 10^{-4}$ | $n_1$ | $k_1$ $(M^{-1}) \times 10^{-6}$ | $n_2$ | $k_2$ $(M^{-1}) \times 10^{-4}$ |
| 0 | †1·6 ± 0·1 | 2·6 ± 0·3 | 0·66 ± 0·06 | 1·60 ± 0·20 | 4·0 ± 0·7 | 2·60 ± 0·70 |
| Glycodeoxycholic | 1·3 ± 0·1 | 3·2 ± 0·3 | — | — | — | — |
| Taurodeoxycholic | 1·8 ± 0·3 | 1·8 ± 0·3 | 0·40 ± 0·04 | 1·90 ± 0·30 | 3·3 ± 0·2 | 3·80 ± 0·50 |
| Cholic | 1·9 ± 0·4 | 1·6 ± 0·4 | 0·31 ± 0·05 | 2·20 ± 0·80 | 3·4 ± 0·2 | 4·30 ± 0·70 |
| Chenodeoxycholic | 2·3 ± 1·1 | 1·3 ± 0·7 | 0·26 ± 0·08 | 0·59 ± 0·22 | 3·9 ± 0·3 | 2·40 ± 0·40 |
| Deoxycholic | 2·3 ± 1·0 | 1·2 ± 0·6 | 0·48 ± 0·08 | 0·80 ± 0·20 | 10·0 ± 6·9 | 0·71 ± 0·60 |

† Estimate of parameter ± asymptotic standard deviation.

Table 2. Effect of various bile acids on the apparent association constant (k) and number of binding sites (n) for sulphadimethoxine and warfarin.

| | Sulphadimethoxine | | Warfarin | | | |
|---|---|---|---|---|---|---|
| Bile acid | n | k $(M^{-1}) \times 10^{-4}$ | $n_1$ | $k_1$ $(M^{-1}) \times 10^{-5}$ | $n_2$ | $k_2$ $(M^{-1}) \times 10^{-3}$ |
| 0 | †1·8 ± 0·1 | 9·5 ± 0·6 | 1·30 ± 0·20 | 1·9 ± 0·7 | 3·8 ± 0·9 | 2·5 ± 0·1 |
| Glycodeoxycholic | 1·7 ± 0·1 | 6·4 ± 0·9 | 1·30 ± 0·10 | 1·3 ± 0·2 | 5·5 ± 0·9 | 1·3 ± 0·4 |
| Taurodeoxycholic | 1·7 ± 0·1 | 3·9 ± 0·5 | 0·80 ± 0·15 | 3·3 ± 1·7 | 3·3 ± 0·2 | 4·4 ± 1·2 |
| Cholic | 1·7 ± 0·1 | 3·9 ± 0·3 | 0·99 ± 0·25 | 1·7 ± 1·0 | 4·0 ± 0·4 | 3·2 ± 1·4 |
| Chenodeoxycholic | 2·0 ± 0·2 | 1·8 ± 0·3 | 0·93 ± 0·25 | 2·3 ± 1·7 | 3·5 ± 0·8 | 2·4 ± 1·7 |
| Deoxycholic | 2·3 ± 0·4 | 1·7 ± 0·4 | 1·10 ± 0·10 | 1·2 ± 0·2 | 1·7 ± 0·1 | 3·0 ± 1·0 |

† Estimate of parameter ± asymptotic standard deviation.

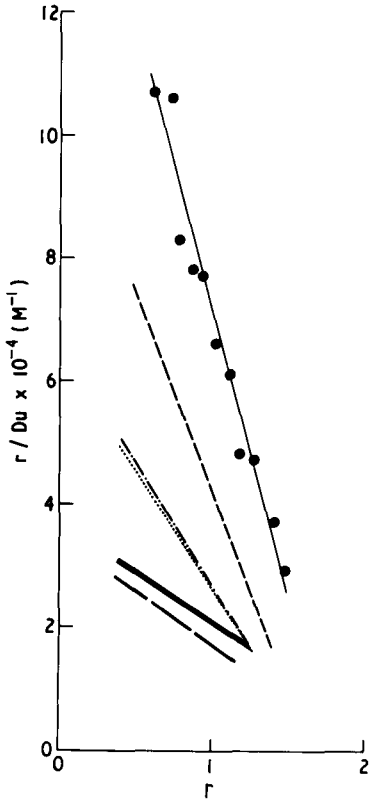


FIG. 2. Scatchard plot for the binding of sulphadimethoxine to 1% w/v HA in the absence (——) and presence of glycodeoxycholic acid (– – – –), taurodeoxycholic acid (·—·—), cholic acid (· · · · ·), deoxycholic acid (▬▬) and chenodeoxycholic acid (— —). Bile acid concentration was 250 μM.

acids were convex. All the bile acids tested displaced warfarin, but they had only small and inconsistent effects upon the binding parameters of warfarin (Table 2). However, it would seem that deoxycholic was the most potent inhibitor of the warfarin–albumin interaction (Table 2) and it increased the unbound fraction of drug by about 2·7 and 1·2-fold at 83 and 833 μM, respectively.

*Discussion*

The values of n and k found in this study for HABA, sulphadimethoxine and warfarin are similar to those quoted in the literature (Oester et al 1976; Vallner 1977; Bowmer & Lindup 1980); but for methyl orange, $k_1$ and $k_2$ are an order of magnitude greater than those reported by Bowmer & Lindup (1980). This discrepancy may be due to differences in buffer composition because chloride ions were present in the phosphate buffer used by Bowmer & Lindup (1980) and these have been reported to reduce the methyl orange–HA interaction (Klotz & Urquhart 1949).

The present work clearly demonstrates that bile acids have the potential to inhibit the binding of some weakly acidic ligands to HA. The dihydroxy bile acids (chenodeoxycholic and deoxycholic) were more potent inhibitors than the trihydroxy acid (cholic) or the bile acid conjugates (glycodeoxycholic and taurodeoxycholic) and this would suggest that increasing the polarity of the bile acid molecule reduces its potency as a binding inhibitor. This is consistent with the observation that the apparent affinity of bile acids for albumin decreases with increasing polarity (Rudman & Kendall 1958; Roda et al 1982). Brock (1976) too, found that the effectiveness of bile acids as inhibitors of the digitoxin–HA interaction diminished with increasing polarity and water solubility. It would be anticipated that the mono-hydroxy bile acid, lithocholic, should be a more potent displacer than the dihydroxy acids used here. Unfortunately we were unable to test this because of the limited solubility of lithocholic acid in phosphate buffer.

Those ligands that were most affected by the presence of bile acids were HABA, methyl orange and sulphadimethoxine and in each case it was mainly the apparent association constant that was reduced. This suggests that for these ligands displacement was brought about by a competitive mechanism. Brock (1976) concluded that competitive inhibition was probably the mechanism by which bile acids displaced digitoxin from HA; but it must be borne in mind that digitoxin has a closer

structural resemblance to bile acids than the ligands used in this study. Moreover, bile acids are anionic detergents (Elworthy et al 1968) and these substances are known to cause conformational changes in the albumin molecule leading to displacement of bound ligands (Steinhardt & Reynolds 1969), so it is conceivable that inhibition of binding may have been due to structural alterations of the HA molecule.

It is interesting that bile acids did not appear to displace phenylbutazone; but they did decrease warfarin binding, albeit less well than the other ligands used. Why phenylbutazone should not be displaced is not clear, especially as phenylbutazone and warfarin are known to compete for albumin binding sites (O'Reilly 1973). Furthermore, phenylbutazone inhibits the binding of sulphonamides to albumin (Anton 1973) and Sudlow et al (1975) suggested that these three ligands all occupy a common binding site(s). Perhaps phenylbutazone has some binding sites shared with warfarin and sulphadimethoxine but which are not available to, or cannot be perturbed by, bile acids.

In conclusion, it would seem that bile acids have the ability to displace some albumin-bound ligands and the mechanism by which this is achieved may be competitive. By themselves, these experiments do not allow any conclusions to be made about whether bile acids may displace highly bound ligands in-vivo. However, Brock (1976) concluded that it was unlikely that bile acids would cause clinically significant displacement of digitoxin in-vivo. Moreover, the normal serum concentration of bile acids is only about 3 μM (Neale et al 1971) and although in patients with liver disease serum bile acid concentrations can be increased 100 times, this is mainly due to a rise in conjugated bile acids (Neale et al 1971) which were not found to be particularly effective inhibitors of binding.

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