Biochemical Pharmacology, Vol. 40, No. 7, pp. 1672–1673, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

Interaction of cimetidine with human serum albumin

(*Received* 12 *April* 1990; *accepted* 31 *May* 1990)

Although clinical studies have provided an estimate of 15–20% for the proportion of protein-bound xenobiotic in the serum of cimetidine-dosed patients [1, 2], no attempt seems to have been made to identify the binding protein. Instead, it has been assumed, as in Ref. 3, that cimetidine interacts with α_1-acid glycoprotein, which is a binding protein present at low concentration but with high affinity for a number of basic drugs [4, 5]. In the present investigation we have adopted the opposing viewpoint that the binding of cimetidine by serum proteins may well be relatively weak, and have therefore studied its interaction with albumin—the plasma protein present at highest concentration and a protein recognized for its ability to form complexes with amphiphilic ligands [6–8].

Materials and Methods

Human serum albumin, bovine serum albumin (both Cohn Fraction V) and cimetidine were used as supplied by the Sigma Chemical Co. (St Louis, MO). Stock solutions of human serum albumin (10–12 mg/mL) and cimetidine (0.3–6 mM) were both prepared by direct dissolution in phosphate–chloride buffer (0.04 M Na_2HPO_4–0.01 M NaH_2PO_4–0.02 M NaCl), pH 7.4, I 0.15. The concentrations of suitably diluted samples were determined spectrophotometrically on the basis of molar absorption coefficents of 36,300 $M^{-1}\,cm^{-1}$ at 280 nm for human serum albumin [9] and 23,250 $M^{-1}\,cm^{-1}$ at 220 nm for cimetidine, a value determined experimentally and confirmed by the manufacturer (Smith Kline and French). For bovine serum albumin the molar absorption coefficient was taken as 43,600 $M^{-1}\,cm^{-1}$ on the basis of a value of 6.6 for $A^{1\%}_{1cm}$ at 280 nm [10].

The interaction between cimetidine and human serum albumin in phosphate–chloride buffer (pH 7.4, I 0.15) was investigated by ultrafiltration [11] at room temperature (26–28°), using an Amicon 8MC ultrafiltration assembly fitted with a YM10 membrane. Mixtures containing albumin (5–6 mg/mL) and cimetidine (0.4–4.0 mM) were prepared by weight from stock solutions of the two reactants. Since preliminary experiments had indicated the occurrence of cimetidine adsorption to the membrane and cell assembly, the cell was pre-equilibrated with mixture to ensure the attainment of adsorption equilibrium prior to ultrafiltration [12]. At that stage a further sample (5 mL) of the test mixture was introduced into the apparatus, pressure (approx. 20 p.s.i.) applied and ultrafiltrate (5–10 drops) collected. This ultrafiltrate was suitably diluted by weight with buffer for spectrophotometric determination of the cimetidine concentration at 220 nm. Measurements at 280 nm were also performed routinely to check for any leakage of protein through the YM10 membrane. Comparable experiments were also performed with mixtures of bovine serum albumin and cimetidine in 0.067 M phosphate, pH 7.9.

The estimates of the cimetidine concentration in the undiluted ultrafiltrate $[S]$ in each reaction mixture with defined total concentrations $[\bar{A}]$ and $[\bar{S}]$ of albumin and cimetidine, respectively, were then used to determine the binding function, ν, for presentation of the results in Scatchard [13] format. However, to avoid the consequences of error distortion associated with linear transforms of the binding equation, the magnitudes of the stoichiometry (n) and intrinsic association constant (K) were obtained by non-linear regression analysis [14] of the (ν, $[S]$) data in terms of the rectangular hyperbolic relationship

$$\nu = ([\bar{S}] - [S])/[\bar{A}] = nK[S]/(1 + K[S]). \quad (1)$$

Results and Discussion

The results of this investigation of the interaction between cimetidine and human serum albumin in phosphate buffer, pH 7.4, I 0.15, are presented (●) as a Scatchard plot in Fig. 1, where the solid line corresponds to the best-fit description obtained by non-linear regression analysis of the untransformed (ν, $[S]$) data in terms of Eqn 1. On that basis the interaction of cimetidine with 4.0 ± 0.8 albumin sites is governed by an intrinsic association constant of 630 ± 160 M^{-1}. For bovine serum albumin under slightly more alkaline conditions (pH 7.9, I 0.2), the corresponding analysis of results (○) signifies the presence of 5.9 ± 1.6 sites with an intrinsic association constant of 260 ± 100 M^{-1} (broken line in Fig. 1). The fact that these estimates are subject to considerable experimental uncertainty (± 2 SE) reflects the difficulties inherent in quantitative characterization of relatively weak interactions. Nevertheless, the results suffice to establish that cimetidine does bind weakly to serum albumin, a phenomenon also detected for other basic drugs such as imipramine and desipramine, for which seven albumin sites with respective K values of 230 and 300 M^{-1} have been reported [4]. In view of the uncertainties associated with evaluation of n it is extremely likely that these basic drugs are all binding to the same albumin sites, i.e. that the various values of n (4, 6, 7) are merely different estimates of the same number of binding sites.

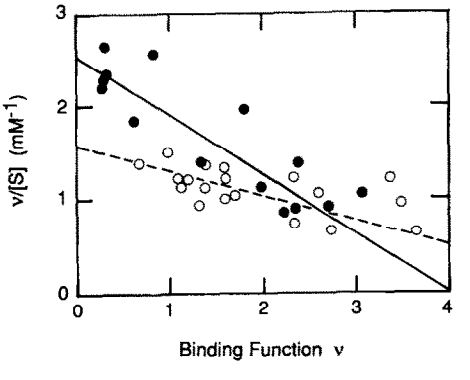

Fig. 1. Scatchard plots of data obtained by ultrafiltration for the interactions of cimetidine with human serum albumin (●) and bovine serum albumin (○) in phosphate buffers (pH 7,4 I 0.15 and pH 7.9, I 0.20, respectively). The lines correspond to the best-fit rectangular hyperbolic relationships obtained by nonlinear regression analysis of the ultransformed (ν, $[S]$) data.

Since the decision to investigate the interaction of cimetidine was made in the belief that such binding might account for the clinical observations [1, 2] that some 15–20% of the drug in human serum is protein-bound, the point at issue is whether an interaction governed by such a small association constant (630 M^{-1}) could assume physiological relevance in the therapeutic situation involving systemic cimetidine concentrations in the low micromolar range [2]. Rearrangement of Eqn 1 yields the relationship

$$([\bar{S}]/[S]) - 1 = nK[\bar{A}]/(1 + K[S]) \cong nK[\bar{A}] \qquad (2)$$

in which the $[S]$ term in the denominator of the right hand side may justifiably be neglected on the grounds that $K[S] \ll 1$ in the systemic environment. From the approximate form of Eqn 2 it is clear that the predicted distribution *in vivo* is only reliant upon the product of n and K, i.e. upon the value of the ordinate intercept in the Scatchard plot. Combination of these estimates of 2.50 and 1.55 mM^{-1} (Fig. 1) with a value of 0.6 mM (40 mg/mL) for the systemic albumin concentration leads to the conclusion that $[S]/[\bar{S}] = 0.4$ and 0.5 for human and bovine serum albumin, respectively. This predicted magnitude of 50–60% for the proportion of albumin-bound xenobiotic is, of course, a maximal estimate inasmuch as it is based on the presumption that cimetidine is the only ligand capable of interaction with the protein sites. Clearly, the functional role of albumin as a transporter of amphiphilic metabolites [6–8] virtually guarantees a situation wherein cimetidine would be only one of several ligands competing for the same albumin sites. Thus, rather than being considered contradictory, the present work and the clinical findings [1, 2] should be regarded as complementary. Whereas the latter establish that a significant proportion (15–20%) of cimetidine is serum bound, the present study points to the likelihood that a relatively weak interaction with albumin accounts for that pharmacological situation. This conclusion is supported by results obtained for lidocaine, a basic drug with strong affinity for α_1-acid glycoprotein but with weak affinity for albumin [15]: for a concentration $[S]$ of 12 μM, the fraction bound in serum is 70%, whereas only 20% is bound by 4% albumin. The correspondence between the latter value and the estimate of 19–20% for protein-bound cimetidine in serum [1, 2] is certainly consistent with the concept that albumin provides the major source of protein-binding for cimetidine in serum.

In summary, ultrafiltration studies have detected the existence of a weak interaction between cimetidine and human serum albumin, a finding supported by corresponding studies with this xenobiotic and bovine serum albumin. Furthermore, the binding characteristics of the interaction with human serum albumin (4 sites, K = 630 M^{-1}) more than suffice to account for the proportion of protein-bound drug in the serum of patients subjected to cimetidine therapy. Thus, although α_1-acid glycoprotein is usually regarded as the specific transporter of basic drugs, the present evidence implicates albumin as the likely binding protein for cimetidine in serum.

Acknowledgement—The support of this investigation by the Australian Research Council is gratefully acknowledged.

Department of Biochemistry
University of Queensland
St Lucia
Queensland 4072
Australia

CRAIG J. WILSON
MARIE A. BOGOYEVITCH
DONALD J. WINZOR

REFERENCES

1. Griffiths R, Lee RM and Taylor DC, Kinetics of cimetidine in man and experimental animals. In: *Cimetidine* (Eds. Burland WL and Simkins MA), pp. 38–53. Excerpta Medica, Amsterdam, 1977.
2. Somogyi A, Rohner H-G and Gugler R, Pharmacokinetics and bioavailability of cimetidine in gastric and duodenal ulcer patients. *Clin Pharmacokinet* **5**: 84–94, 1980.
3. Parish RC, Gotz VP and Mehta JL, Cimetidine does not increase α_1-acid glycoprotein serum concentrations. *Br J Clin Pharmacol* **25**: 514–517, 1988.
4. Javaid JA, Kendricks K and Davis JM, α_1-Acid glycoprotein involvement in high affinity binding of tricyclic antidepressants to human plasma. *Biochem Pharmacol* **32**: 1149–1153, 1983.
5. Müller WE and Stilbauer AE, Characterization of a common binding site for basic drugs on human α_1-acid glycoprotein (orosomucoid). *Naunyn-Schmiedebergs Arch Pharmacol* **322**; 170–173, 1983.
6. Peters T and Read RG, Serum albumin as a transport protein. In: *Transport by Proteins* (Eds. Blauer G and Sund H), pp. 57–73. de Gruyter, Berlin, 1978.
7. Routledge PA, The plasma protein binding of basic drugs. *Br J Clin Pharmacol* **22**: 499–506, 1986.
8. Kremer JMH, Wilting J and Janseen LHM, Drug binding to human α_1-acid glycoprotein in health and disease. *Pharmacol Rev* **40**: 1–47, 1988.
9. Hogg PJ and Winzor DJ, Evidence for the preferential interaction of micellar chlorpromazine with human serum albumin. *Biochem Pharmacol* **33**: 1998–1999, 1984.
10. Kronman MJ and Foster JF, Sedimentation and optical rotation behavior of bovine plasma albumin at low pH in the presence of various anions. Effects of charge on molecular expansion. *Arch Biochem Biophys* **72**: 205–218, 1957.
11. Sophianopoulos JA, Durham SJ, Sophianopoulos AJ, Ragsdale HL and Cropper WJ, Ultrafiltration is theoretically equivalent to equilibrium dialysis but much simpler to carry out. *Arch Biochem Biophys* **187**: 132–137, 1978.
12. Harris SJ and Winzor DJ, Graphical analysis of binding data reflecting competition between two ligands for the same acceptor sites. *Anal Biochem* **169**: 319–327, 1988.
13. Scatchard G, The attractions of proteins for small molecules and ions. *Ann NY Acad Sci* **51**: 660–672, 1949.
14. Duggleby RG, A nonlinear regression analysis program for small computers. *Anal Biochem* **110**: 9–18, 1981.
15. Routledge PA, Barchowsky A, Bjornsson TD, Kitchell BB and Shand DG, Lidocaine plasma protein binding. *Clin Pharmacol Ther* **27**: 347–351, 1980.