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Interactions of Drugs with Proteins I: Binding of Tricyclic Thymoleptics to Human and Bovine Plasma Proteins

H. J. WEDER* and M. H. BICKEL

Abstract □ Data on the binding of imipramine, desipramine, and 3-chlorodesmethylimipramine to plasma proteins have been obtained over a wide range of ligand concentration using a modified equilibrium dialysis technique. Plasma proteins other than albumin do not appreciably contribute to complex formation with the drugs studied. Fifty-nine percent imipramine is bound to albumin in the plasma level range reached under therapeutic conditions. However, the association constants of the complex is low. Species differences in the binding capacity of albumin were observed. Apparently, atypical binding behavior was disclosed for desipramine and 3-chlorodesmethylimipramine. A binding model is discussed, and values of binding parameters are given.

Keyphrases □ Thymoleptics, tricyclic—binding to human, bovine plasma proteins □ Proteins, human, bovine plasma—imipramine, desipramine, 3-chlorodesmethylimipramine, interaction, binding, model

These studies on the binding of drugs and other compounds to plasma proteins are aimed at gaining insight into the following problems:

1. Possible influence on the pharmacokinetics of a drug by its interactions with proteins.
2. Mechanism of interactions between drugs or model compounds and proteins and physicochemical interpretation.
3. Critique of methods used in the study of drug–protein interactions.

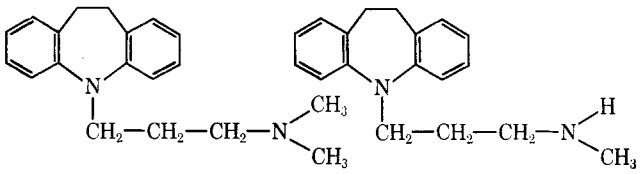
Part I contains data on the complex formation of imipramine, its active metabolite desipramine (desmethylimipramine), and 3-chlorodesmethylimipramine with bovine albumin and human albumin, γ-globulin, plasma, and serum in concentration ranges including the one met with under therapeutic conditions. The first two drugs are used clinically; the latter is a metabolite of the clinically used 3-chloroimipramine accumulating in rats (1) and human (2).

Imipramine (IP) is one of the few drugs about which much of the metabolic and pharmacokinetic data is known (3–8). In addition, physicochemical data of all its major metabolites have been reported (9). Sensitive methods exist for determining imipramine, desipramine, and 3-chlorodesmethylimipramine (5, 6, 10, 11).

Data on IP binding to plasma proteins were published by Tinao and Gomez-Guillen (12) in 1963. Data and species differences in the binding of desipramine to plasma proteins have recently been reported by Borgå *et al.* (13). Earlier, Gillette (14, 15) reported on the binding of imipramine to liver microsomes, which presumably is the reason for the high concentrations of imipramine and related drugs in lung, liver, or kidney tissue in the rat *in vivo* (5, 8). Beside many reports on interactions between thymoleptics and membranes [reviewed by Glowinski and Baldessarini (16)], an interaction between imipramine and the outer membrane of blood platelets has also been reported (17).

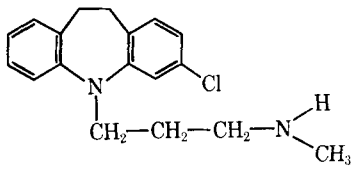
EXPERIMENTAL

Materials—The hydrochlorides of imipramine,[1] desipramine[1] (DMI), and 3-chlorodesmethylimipramine[1] (CDMI) were used.



imipramine
I

desipramine
II



3-chlorodesmethylimipramine
III

Also used were 10,11-^{14}C-imipramine hydrochloride[2] (8.05 mc./mmole) and ^{3}H-acetic anhydride[2] (in benzene, 500 mc./mmole). Gas chromatographic (11) and spectrophotometric tests showed

[1] Supplied by Geigy Ltd., Basel, Switzerland.
[2] Acquired from Radiochemical Centre, Amersham, England.

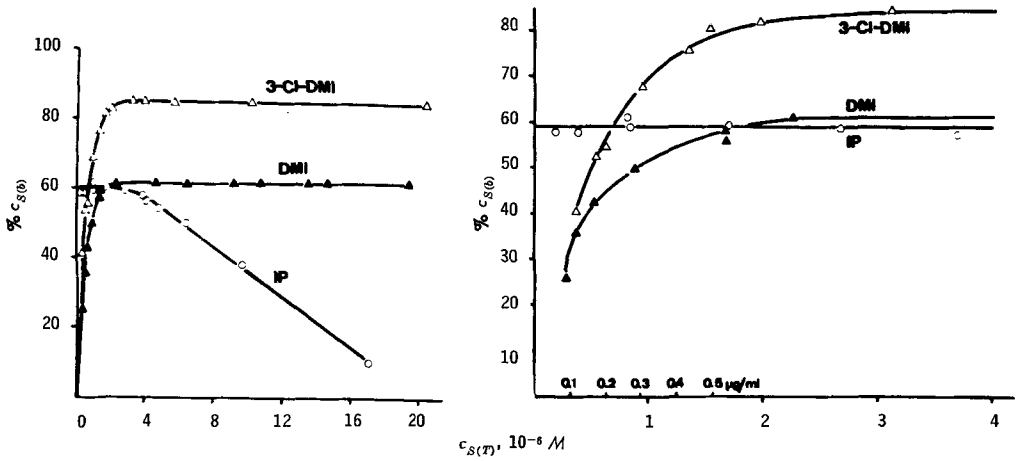


Figure 1—*Fraction of IP, DMI, and CDMI bound to HA, pH 7.4, 20°. HA 4% = $6.15 \cdot 10^{-4}$ M. % $c_{S(b)}$ = fraction of ligand bound. $c_{S(T)}$ = total molar ligand concentration.*

purities of >99% for all compounds. The radiochemical purities were >98%.

Demineralized crystallized bovine albumin[3] (BA), electrophoretically pure human albumin[4] (HA), and γ-globulin[4] (HGLO) were used. All reagents used were of analytical purity.

Normal human plasma (NHP) and serum (NHS) were pooled and stored at −15° for no longer than 1 week.

The ligands (IP, DMI, and CDMI) were dissolved in 0.01 *M* phosphate buffer containing 0.9% NaCl, at pH 7.4 ± 0.05 and ionic strength of 0.19. The proteins (BA, HA, and HGLO) were dissolved in the same medium by gentle agitation.

Methods—Unbound and total ligand concentrations were determined after equilibrium dialysis. The dialysis chamber was developed in this laboratory and will be described in a subsequent paper (18).

Analytical Methods—^{14}C-IP in aqueous solutions was measured by direct liquid scintillation counting. Protein quenching was corrected for by the channel ratio method. DMI and CDMI were determined by the isotope derivative method described by Hammer and Brodie (10). In all cases, a Packard Tri-Carb 314 E scintillation spectrometer and the following scintillation system were used: 4 g./l. 2,5-bis-[5′-*tert*-butylbenzoxazolyl(2′)]-thiophene (BBOT) and 40 g./l. naphthalene in dioxane–xylene–ethyleneglycol monomethyl ether (3:1:3, v/v).

RESULTS

Table I shows the fraction of the total concentrations of IP, DMI, and CDMI bound to HA, HGLO, BA, serum, and plasma. The values for two total concentrations are given for each ligand, the first concentration representing the range of plasma levels observed in therapy (2, 19, 20). The albumin concentrations of the sera showed a mean value of 4.1%.

The fractions of IP, DMI, and CDMI bound in a 4% HA solution as a function of total ligand concentration in the range of 0.1–20 µ*M* are depicted in Fig. 1. Whereas a typical curve results with IP, the curves obtained with DMI and CDMI represent apparently atypical cases.

IP in the concentration range of 0.2 µ*M* (0.06 mcg./ml.)–3 µ*M* (0.95 mcg./ml.), *i.e.*, in the therapeutic range, is bound at a fraction of 58.9 ± 2.8%. A further increase of the total ligand concentration up to 17.2 µ*M* (54.5 mcg./ml.) leads to a gradual decrease to a bound fraction of 10.5 ± 1.9%.

The DMI fraction bound shows a sharp increase from 23.5 ± 2.1% to 61.2 ± 2.4% in the (therapeutic) concentration range 0.27 µ*M* (0.08 mcg./ml.)–3 µ*M* (0.91 mcg./ml.). With a further increase to 19.3 µ*M* (5.85 mcg./ml.), the fraction bound remains constant.

An analogous atypical curve is obtained with CDMI; from 35.2 ± 1.8% to 84.2 ± 3.1% are bound in the range of 0.3 µ*M* (0.1 mcg./ml.)–3 µ*M* (1.0 mcg./ml.), the fraction bound again remaining constant at higher concentrations. Thus, 3 µ*M* represents a critical concentration in the atypical cases of DMI and CDMI.

DISCUSSION

An interaction between albumin and the ligands IP, DMI, and CDMI is demonstrated by the results. The experiments did not reveal an influence on the binding affinity of HA by other plasma proteins. It must be concluded that albumin is the only complexing partner for these ligands in plasma. Species differences are observed for all three ligands, the binding affinity being significantly higher with bovine than with HA.

The therapeutic treatment of patients with IP or DMI leads to plasma levels of 0.005–0.3 mcg./ml. (2, 19, 20). Similar concentrations are observed with other tricyclic drugs, although the absolute values are very low compared with many other classes of drugs. According to the results, up to 70% of IP, DMI, and CDMI in plasma are bound to albumin in the therapeutic concentration range, leaving a free concentration of 30% or more. These data are of little help for the estimation of a possible influence on the pharmacokinetics and pharmacodynamics of the drugs by their interactions with plasma albumin, In this respect, a far better key is provided by the absolute values of the binding affinity, *i.e.*, the association constant, because only the stability of these complexes can possibly be related with pharmacological phenomena. Therefore, information should be obtained on the main forces responsible for drug–protein complexes and not merely on their stabilizing factors.

The following model allows the estimation of the association constant of the IP–HA complex under the experimental conditions used. The c_p ($6.15 \cdot 10^{-4}$ *M*) is the concentration of albumin *P*, where each molecule *P* can bind *n* ligand molecules *S*. Thus *P* has *n* binding sites. If all the *n* binding sites are occupied with ligands, the total binding capacity per liter solution is $c_p \cdot n$. This capacity, however, can only be reached if the concentration of the free ligands, $c_{S(f)}$, is infinite. With relatively low ligand concentrations, the binding sites are only partially occupied in a statistical manner. A correlation between the number of occupied and unoccupied binding sites can be derived under the assumption that the reactivity of all binding sites is equal and that no interactions occur between binding sites. If *A* is a binding site of albumin, *S* a ligand molecule, and *AS* an occupied binding site, then the association or affinity constant *k* for the binding reaction $A + S \rightleftharpoons AS$ is

$$k = \frac{c_{AS}}{c_A \cdot c_{S(f)}} \qquad \text{(Eq. 1)}$$

If, under the experimental conditions, *r* out of *n* binding sites are occupied, then

$$c_{AS} = c_p \cdot r \qquad \text{(Eq. 2)}$$

and

$$c_A = c_p \cdot n - c_p \cdot r \qquad \text{(Eq. 3)}$$

Thus, Eq. 1 becomes

$$k = \frac{c_p \cdot r}{(c_p \cdot n - c_p \cdot r) c_{S(f)}} \qquad \text{(Eq. 4)}$$

[3] Acquired from Poviet Producten N.V., Amsterdam, The Netherlands.

[4] Supplied by the Central Laboratories, Swiss Red Cross, Berne, Switzerland.

Table I—Percent of Total Concentration IP, DMI, and CDMI Bound to Plasma Proteins[a]

Macromolecular System Protein Concn.		Total Concentration IP 100% = 0.2 μM = 0.063 mcg./ml.	IP 100% = 10 μM = 3.16 mcg./ml.	DMI 100% = 0.3 μM = 0.091 mcg./ml.	DMI 100% = 10 μM = 3.02 mcg./ml.	DCMI 100% = 0.3 μM = 0.101 mcg./ml.	DCMI 100% = 10 μM = 3.37 mcg./ml.
BA	4%	72.3 ± 3.6	45.9 ± 2.0	35.5 ± 1.8	75.9 ± 3.4	46.1 ± 1.8	90.2 ± 4.2
HA	4%	60.0 ± 1.2	36.4 ± 1.3	28.3 ± 2.0	62.3 ± 2.9	37.5 ± 1.9	83.3 ± 3.8
HGLO	0.7%	0	<1	0	<1	0	<1
HA + HGLO	4% + 0.7%	58.9 ± 2.1	38.2 ± 1.4	30.3 ± 2.4	65.1 ± 3.2	40.1 ± 2.3	82.7 ± 3.6
NHS		64.7 ± 2.8	40.0 ± 1.2	32.7 ± 2.1	68.4 ± 3.4	35.3 ± 2.0	85.2 ± 4.0
NHP		59.1 ± 2.6	37.7 ± 1.8	30.8 ± 2.5	61.2 ± 3.1	38.6 ± 1.8	81.8 ± 4.1

[a] pH 7.4, 20°, ± *SD* (6–10 experiments). For abbreviations, see *Experimental.*

or

$$c_p \cdot r = \frac{c_p \cdot n}{1 + [1/(k \cdot c_{S(f)})]} \qquad \text{(Eq. 5)}$$

Figure 2 shows the plot of the free ligand concentration $c_{S(f)}$ against the total ligand concentration $c_{S(T)} = c_p \cdot r + c_{S(f)}$. A curve is thereby obtained for each albumin complex with the three ligands used. Curves 4 ($k = 0$) and 5 ($k = \infty$) represent the cases of total lack and of infinitely high binding affinity, respectively. Thus, the sequence of the association constants for the complexes in the concentration range 1–11 μM is $k_{CDMI} > k_{DMI} > k_{IP}$. Only IP fits the binding model introduced, whereas the atypically behaving DMI and CDMI do not. However, with all three ligands, no deviation from the ideal binding curve can be observed in the lowest concentration range (0 – 1 μM). In this range the sequence of binding affinities is $k_{IP} > k_{CDMI} > k_{DMI}$ (Table I).

Graphical determination of the binding capacity of HA for IP leads to a value of about 5.1 μM (Fig. 2). By calculation, an association constant of $k_{IP} = 0.5 \cdot 10^4\ M^{-1}$ is obtained. According to Fig. 2 the binding capacities of albumin for DMI and CDMI must be much higher than for IP. A hypothetical explanation of the atypical curves of DMI and CDMI could be a conformational change of the protein by a primary interaction, which then results in an increase of available binding sites, possibly of higher reactivity.

It is unlikely that at therapeutic concentrations the binding of IP to plasma albumin exerts an appreciable influence on pharmacokinetics and thus pharmacodynamics, since the association constant of the IP–HA complex is less than $10^4\ M^{-1}$ (21, 22).

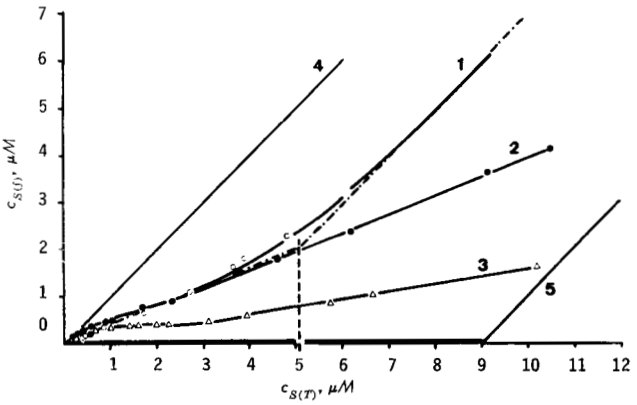


Figure 2—*Free ligand concentration* $c_{S(f)}$ *as a function of total concentration* $c_{S(T)}$. *For conditions, see Fig. 1. Key: 1 = IP, 2 = DMI, 3 = CDMI, and 4 and 5 = theoretical binding curves for* k = 0 *and* k = ∞, *respectively.*

Further results on the binding mechanism as well as thermodynamic data will be presented and discussed in a subsequent report (18).

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* Present address: Institute of Molecular Biology and Biophysics, Federal Institute of Technology, Zurich, Switzerland.