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BINDING OF CICLAZINDOL BY HUMAN PLASMA INVOLVES THE GLYCOPROTEIN FRACTION

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Ciclazindol, 10-(m-chlorophenyl)-2,3,4,10-tetrahydropyrimido(1,2)indol-10-ol hydrochloride (Fig. 1) is a drug of novel structure that may prove useful as an antidepressant (1). For certain tricyclic antidepressant drugs, correlations between plasma concentrations and pharmacological effects have been established (2,3) and it has been suggested that such relationships may be useful in adjusting dosage to give maximum response (4). However, since it is thought that only the unbound fraction of the drug can elicit a response, it is important to consider the degree of binding of the drug to plasma proteins (5,6). It is also of use, in certain cases, to establish both the protein to which the drug is bound and the strength of such binding, since it may then be possible to predict interaction effects, such as the displacement of bound phenylbutazone by co-administered albumin-bound drugs (7). Furthermore, the influence of the binding on the disposition of the drug may also be assessed (8).

This report concerns the binding of ciclazindol by whole human plasma and by isolated fractions of plasma. A novel development of the usual equilibrium dialysis technique is also described, this yields a measure of the overall strength of binding of drugs to proteins and plasma.

Equilibrium dialysis was carried out in 2 ml perspex cells separated into half-cells by $3cm^2$ of semipermeable membrane (Spectrapor grade 2, MSE Ltd., Sussex). Buffer (0.1M phosphate/HCl, pH 7.4) containing the appropriate amounts of {2-^{14}C} ciclazindol (sp. act. 4.08 μCi/mg) was placed in one half-cell and plasma or protein solution in the other. After shaking at 37° for 3h the contents of the half-cells were removed and radioactivity measured by liquid scintillation counting using NE260 scintillant (Nuclear Enterprises

Ltd., Edinburgh). The plasma used in all experiments was obtained from healthy male volunteers.

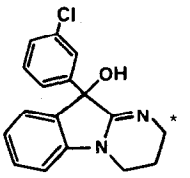

Fig 1. Structural formula of Ciclazindol:
* denotes position of ^{14}C label

The extent of binding of ciclazindol by whole human plasma was investigated over a range of drug concentrations. The results (Table 1) show that ciclazindol was extensively bound by human plasma at concentrations below 10^{-4}M. Furthermore, over the concentration range that appears to be useful in treating depression {3 x 10^{-6}M to 1.5 x 10^{-5}M (Guz, unpublished observations} there was no significant change in the degree of binding. A rise in the drug concentration, as a result of increased dose for example, is, therefore, unlikely to result in a disproportionate increase in available (unbound) drug.

Table 1 BINDING OF CICLAZINDOL IN WHOLE PLASMA

CICLAZINDOL CONCENTRATION	% Bound	S.E.M.	N
3.0 x 10^{-4}M	67.4	0.5	6
3.6 x 10^{-5}M	80.2	0.6	6
7.6 x 10^{-6}M	86.3	0.3	6
1.3 x 10^{-6}M	88.9	0.3	6
1.9 x 10^{-7}M	86.9	0.3	6

The binding of ciclazindol was also investigated at a single concentration (3 x 10^{-6}M) in the following subfractions of human plasma: Albumin (essentially fatty-acid free) (Sigma Ltd., London), α-globulin (Fraction IV), β-globulin (Fraction III), γ-globulin (Fraction II), fibrinogen (Fraction I), transferrin, IgG and glycoprotein (Fraction VI) (all from Miles Laboratories Ltd., Bucks) and β-lipoprotein (ICN, obtained through Uniscience Ltd., London). The results (Table II) show that although ciclazindol was bound by albumin, the binding was not as extensive as that by whole plasma. However, extensive binding was seen with Fraction VI, the glycoprotein fraction, indicating that a component of this mixture of proteins may be responsible for the binding in plasma.

Table II THE BINDING OF CICLAZINDOL ($3x10^{-6}$M) TO SUBFRACTIONS OF HUMAN PLASMA

SUBFRACTION	% Bound	S.E.M.	N
Albumin (4%(w/v))	58.5	0.3	3
α-Globulin (0.81%(w/v))	17.2	1.4	5
β-Globulin (0.79%(w/v))	10.4	0.6	10
γ-Globulin (0.74%(w/v))	1.8	0.7	10
Fibrinogen (0.34%(w/v))	8.6	0.9	5
Transferin (0.25%(w/v))	5.7	0.6	4
IgG (0.15%(w/v))	1.8	0.4	3
Glycoprotein (0.15%(w/v))	74.5	0.7	3
β-Lipoprotein (0.34%(w/v))	6.4	1.1	4

All protein fractions were dissolved in 0.1M phosphate/HCl buffer pH 7.4 at approximately physiological concentrations (Documenta Geigy, 1972)

Attempts to derive an association constant for the binding of ciclazindol in whole plasma by reciprocal or Scatchard analysis resulted in curved plots. A method was therefore developed whereby the overall strength of binding could be assessed. In this technique, similar plasma samples or protein solutions were placed in both half-cells of the dialysis apparatus and the drug was added to one side. The concentrations of ciclazindol in both half-cells was then measured in groups of cells at various times until equilibrium was reached. The results gave the time-course of equilibration of the drug within the system. A plot of $\log_{10}$ of the concentration gradient against t gave a straight line whose slope could be described in terms of a half-life.

Experiments of this type were carried out for ciclazindol in plasma, albumin and the glycoprotein fraction. The results are shown in Table III.

Table III HALF-LIVES OF EQUILIBRATION OF CICLAZINDOL

SYSTEM	HALF-LIFE (h)
Buffer alone	0.3
Whole plasma	10.5
4% (w/v) albumin	0.6
0.15% (w/v) fraction VI	2.1

It appears from these results that the strength of binding of ciclazindol to albumin was considerably less than that either to whole plasma or to the glycoprotein fraction. Although ciclazindol did not appear to be bound as avidly by the glycoprotein fraction as by plasma, it is possible that the binding site(s) on the glycoprotein may have been adversely affected during isolation.

Further evidence that a component of the glycoprotein fraction was responsible for the strong binding of ciclazindol in plasma was obtained by comparing the relative affinities of albumin, glycoprotein and plasma for the drug. The drug was added to a dialysis cell containing different proteins in each half-cell. After incubation for 24h the concentration

of ciclazindol in the two half-cells was determined and the ratio between them calculated (see Table IV).

Table IV DISTRIBUTION OF CICLAZINDOL BETWEEN DIFFERENT PROTEIN SOLUTIONS AT EQUILIBRIUM

COMPARISON	RATIO	S.E.M.	N
Glycoprotein/Albumin	2.56	0.08	7
Plasma/Albumin	2.58	0.03	8
Glycoprotein/Plasma	1.19	0.04	8

The similarity in the affinities of both plasma and the glycoprotein fraction for ciclazindol, suggested by a distribution ratio of one, further indicates that a component of fraction VI may be responsible for the binding in plasma. Furthermore, the relatively minor contribution of albumin binding was confirmed by the extensive accumulation of the drug by both plasma and fraction VI when compared with albumin.

Few reports of drugs that bind to proteins of fraction VI have appeared in the literature (9). It is, therefore, somewhat difficult to speculate on the possible clinical implications of such binding. The possibility of drug interactions cannot, however, be excluded.

Work is in progress to determine the component of fraction VI to which ciclazindol is bound and to evaluate the significance of this binding.

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