PRAZOSIN PROTEIN BINDING IN HEALTH AND DISEASE

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- 1 Experiments have been performed using equilibrium dialysis to determine the binding of [^{14}C]-prazosin to albumin, to α_1 -acid glycoprotein and to the plasma proteins of normal subjects and of patients with cirrhosis, chronic renal failure or chronic heart failure.
- 2 The influence of propranolol on prazosin binding has been studied. In addition, red blood cell to plasma partitioning of prazosin has been quantified.
- 3 The dissociation constant for prazosin binding to albumin was $3 \times 10^{-5}\text{M}$ and to α_1 -acid glycoprotein was $1.9 \times 10^{-6}\text{M}$.
- 4 In fourteen normal subjects the free fraction of prazosin was 0.051 ± 0.007 .
- 5 In seven patients with cirrhosis free fraction of prazosin was 0.064 ± 0.017 ($P < 0.05$ compared to normal). In nine patients with chronic renal failure the free fraction was 0.077 ± 0.033 ($P < 0.05$) and in eight congestive heart failure patients the value was 0.064 ± 0.027 ($P > 0.05$).
- 6 The range of prazosin free fraction was substantially greater in the patients than in normal subjects. In cirrhotic patients free fraction of prazosin correlated significantly ($r = -0.92$) with albumin concentration.
- 7 Propranolol did not influence prazosin protein binding. In blood 20% of prazosin is associated with red cells.
- 8 The considerably greater range of prazosin free fraction in the patients suggests that caution should be used when prescribing the drug for subjects with these conditions. Both albumin and the acute phase reactant α_1 -acid glycoprotein bind prazosin *in vitro*.

Introduction

A generally accepted and applicable principle is that the intensity of a pharmacological response to a drug is more closely related to the concentration of free drug in plasma than to total drug concentration (Koch-Weser & Sellers, 1976). This is specially relevant to drugs which ordinarily are highly bound to plasma proteins within the therapeutic concentration range, since many of these drugs are subject to changes in plasma binding caused by disease states or drug interactions which can produce substantial increases in free concentrations (Koch-Weser & Sellers, 1976).

The hypotensive agent prazosin is extensively

bound to proteins in normal human plasma (Hobbs, Twomey & Palmer, 1978). Although prazosin is often used in conditions where quantitative or qualitative changes in proteins might occur, no data are currently available on the influence of such disease states on the plasma protein binding of this drug. Nor is there any information on possible displacement of prazosin from proteins by other drugs. The studies described here were designed to investigate prazosin protein binding in patients with cirrhosis, congestive heart failure and chronic renal failure, and to examine the influence of propranolol on prazosin protein binding. The affinity of prazosin for albumin has been determined and, because prazosin is a basic drug, affinity for α_1 -acid glycoprotein was also assessed. In addition, the red blood cell to plasma partitioning has been defined.

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Methods

2-[^{14}C]-prazosin HCl of specific activity 53 $\mu\text{Ci}/\mu\text{mol}$ was supplied by Pfizer Inc., Groton, Conn. The radiochemical purity of this substance was demonstrated to be greater than 99% by two thin layer chromatography systems.

Blood for protein binding studies was drawn through regular needles into plastic syringes, transferred to heparinized Venoject® tubes, centrifuged and the plasma stored at -20°C . Preliminary studies had demonstrated that freezing did not change the protein binding of prazosin. In order to determine binding in normal subjects, plasma was obtained from fourteen healthy people aged 25–35 years (seven men and seven women). For the study of prazosin protein binding in disease states, plasma was obtained from eight subjects with chronic congestive heart failure, nine with chronic renal failure and seven with biopsy proven hepatic cirrhosis. The heart failure patients were all classified as being in stage III or IV failure according to New York Heart Association criteria. Their range of albumin concentration was 35 g/l to 44 g/l, with a mean value of 40.8 g/l. The chronic renal failure patients had albumin concentrations from 33 g/l to 49 g/l, the mean value being 41 g/l. Creatinine concentrations ranged from 79.6 $\mu\text{mol/l}$ to 1238 $\mu\text{mol/l}$ with a mean of 817 $\mu\text{mol/l}$. Five of the renal failure patients were maintained without dialysis. In the remaining four, blood for protein binding was drawn on a non-dialysis day. In the subjects with cirrhosis, albumin concentrations ranged from 26 g/l to 42 g/l, mean 36 g/l. At the time when blood for protein binding studies was drawn the drug therapy of the patients was as follows. Congestive heart failure: digoxin (7), frusemide (2), aminophylline (1), allopurinol (1), no drug (1); chronic renal failure: iron, folic acid and vitamins (8), propranolol (7), methyldopa (5), hydralazine (4), frusemide (2), digoxin (2), no drug (1); none of the cirrhotic patients had received any drugs for 48 h before blood was drawn. The normal subjects were receiving no drugs.

Plasma protein binding experiments were performed using a Dianorm® equilibrium dialysis system at 37°C . One side of the dialysis cell contained pH 7.4 Krebs buffer equal in volume to plasma contained on the opposite side of a cellulose membrane (Spectrum Medical, Gardena, California). [^{14}C]-prazosin was added to the buffer compartment in the initial concentration of 78 nmol/l (30 ng/ml). The concentration of radioactivity at equilibrium in each compartment was determined by scintillation counting and the free fraction was determined by dividing the $\text{d min}^{-1} \text{ ml}^{-1}$ on the buffer side by the $\text{d min}^{-1} \text{ ml}^{-1}$ on the protein side. Preliminary experiments with [^{14}C]-prazosin demonstrated that

equilibrium between plasma and buffer was reached within 2 h of the beginning of the dialysis. In the studies reported here, dialysis was carried out for 4 h.

In order to determine dissociation constants for prazosin binding to albumin and α_1 -acid glycoprotein equilibrium dialysis was used as described above. Human albumin and human α_1 -glycoprotein (both obtained from Sigma Chemical Co., St. Louis, MO.) were dissolved in pH 7.4 Krebs buffer. Preliminary experiments revealed that with both albumin and α_1 -acid glycoprotein the percentage binding of prazosin was constant from very low concentrations up to the limit of prazosin solubility in Krebs buffer at 37°C (131 $\mu\text{mol/l}$). In order to permit determination of dissociation constants the concentration of prazosin was kept constant at 78 nmol/l and the concentration of protein was varied. The range of albumin concentration used was 8.8 $\mu\text{mol/l}$ to 677 $\mu\text{mol/l}$ (0.57 g/l to 44 g/l) and the range of α_1 -acid glycoprotein used was 6.8 $\mu\text{mol/l}$ to 20.4 $\mu\text{mol/l}$ (0.3 g/l to 0.9 g/l). Dissociation constants were then calculated by the method recently described by Romer & Bickel (1979) using the Scatchard relationship:

$$\frac{\text{Lb}}{\text{Bo}} = \text{KLbmax} - \text{KLb}$$

where Lb is concentration of bound ligand, Bo is total binder concentration and K is the association constant. It should be noted that in this form of the relationship, protein is the ligand and drug is treated as binder. Results are expressed as the dissociation constant Kd, which is the reciprocal of K.

In order to assess the influence on prazosin protein binding of propranolol, another basic drug commonly prescribed with prazosin, binding in normal plasma was performed in the presence of propranolol concentrations ranging from 96 nmol/l to 3.8 $\mu\text{mol/l}$, which spans the concentration commonly observed after the therapeutic use of propranolol.

Red blood cell to plasma partitioning studies were performed on 20 ml of fresh blood which was immediately placed in a glass flask containing a small amount of heparin, treated to reduce clotting (Siliclad) and maintained at 37°C in a shaking water bath. [^{14}C]-prazosin was added to this blood in order to achieve a concentration of 78 nmol/l. Samples were drawn from this blood at 1, 5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min. At each sampling time an aliquot of whole blood was added to digestion fluid for scintillation counting and a second aliquot was briefly centrifuged (approximately 30 s) and a portion of the plasma used for scintillation counting. The haematocrit was measured at the beginning and end of each incubation period. Partitioning of prazosin

Figure 1 (a) Binding of $[^{14}\text{C}]$ -prazosin to human albumin with prazosin concentration held constant. Each data point is the mean of two determinations. $\text{Lb} = [\text{Prazosin}]$ bound, $\text{Bo} = [\text{albumin}]$, $r = -0.99$. (b) Binding of $[^{14}\text{C}]$ -prazosin to human α_1 -acid glycoprotein with prazosin concentration held constant. Each data point is the mean of two determinations. $\text{Lb} = [\text{Prazosin}]$ bound, $\text{Bo} = [\alpha_1\text{-acid glycoprotein}]$, $r = -0.97$.

between plasma and red blood cells (α_{bc}) was calculated from the expression:

$$\lambda_{bc} = 1 - \frac{C_p}{C_b} (1 - H)$$

where C_p and C_b are prazosin concentrations ($\text{d min}^{-1} \text{ ml}^{-1}$) in plasma and whole blood respectively and H is the haematocrit expressed as a fraction. λ_{bc} is a term which indicates the fraction of drug in a specimen of whole blood which is bound to or within the red cells.

Results are expressed as mean $\pm$ s.d. and statistical analysis was by paired or unpaired t -test as appropriate.

Results

Binding constants

The binding of prazosin to human albumin is shown in Figure 1a, and to human glycoprotein in Figure 1b. The dissociation constant for prazosin binding to albumin is $3 \times 10^{-5} \text{ M}$ and to α_1 -acid glycoprotein is $1.9 \times 10^{-6} \text{ M}$.

Normal subjects

In fourteen normal subjects the free fraction of prazosin was 0.051 ± 0.007 . In seven male subjects the free fraction was 0.049 ± 0.05 and in seven females 0.053 ± 0.09 ($P = 0.3$).

Disease states

Table 1 shows the influence of certain disease states on prazosin free fraction. In patients with cirrhosis there was a highly significant linear correlation between albumin concentration and free fraction of prazosin (Figure 2). Congestive heart failure and chronic renal failure patients showed no significant correlations between these two parameters. Similarly, there was no correlation between prazosin free fraction and serum creatinine concentrations in chronic renal failure patients.

Prazosin binding in the presence of propranolol

The free fraction of prazosin remained unchanged in normal plasma over the propranolol concentration range $0\text{--}3.8 \mu\text{mol/l}$.

Table 1 Protein binding of Prazosin in disease states

	Free fraction	Range	Significance level compared to normal (unpaired t -test)
Normal ($n = 14$)	0.05 ± 0.007	$0.042\text{--}0.067$	
Cirrhosis ($n = 7$)	0.064 ± 0.017	$0.046\text{--}0.092$	$P < 0.05$
Chronic renal failure ($n = 9$)	0.077 ± 0.033	$0.05\text{--}0.15$	$P < 0.05$
Congestive heart failure ($n = 8$)	0.064 ± 0.027	$0.04\text{--}0.13$	$P > 0.05$

Figure 2 Free fraction of [^{14}C]-prazosin in seven subjects with cirrhosis. $r = -0.92$, $P < 0.005$.

Prazosin partitioning between plasma and red cells

Equilibrium between red cells and plasma was achieved in less than 60 s. At equilibrium the red blood cell to plasma partition coefficient was 0.2.

Discussion

The affinity of prazosin for human plasma proteins has not previously been quantified. Attempts to define dissociation constants by increasing prazosin concentration with protein concentration kept constant led to the discovery that both with albumin and α_1 -acid glycoprotein, free fraction did not change up to the limit of prazosin solubility. In this respect prazosin was behaving in a way similar to other basic drugs, protein binding having been shown to be independent of drug concentration for tricyclic (Borga, Azarnoff, Forskell & Sjöqvist, 1969) amphetamine (Franksson & Arggare, 1970) and methadone (Olsen, 1972). When the protein concentration is kept constant in this way, a Scatchard plot of r/D vs r (where r is the number of moles of drug bound per mole of protein, and D is the concentration of unbound drug) results in a curved line. This again is similar to the findings with methadone (Olsen, 1972) and prevents calculation of the dissociation constant. In view of this observation, the binding studies were repeated with the prazosin concentration held constant while the protein concentration was varied but always considerably exceeded the drug concentration. The theoretical basis for the analysis of data generated by such a technique has recently been presented (Romer & Bickel 1979). Using this method, the dissociation constant for prazosin binding to albumin was found to be $3 \times 10^{-5} \text{ M}$ and to α_1 -acid glycoprotein

$1.9 \times 10^{-6} \text{ M}$. Thus prazosin, a basic drug with a pK_a of 6.5, has a 16 fold greater affinity for the acidic α_1 -glycoprotein than for albumin. Considerable attention had been devoted to the possible role of α_1 -acid glycoprotein in the binding of basic drugs since the finding that quinidine free fraction decreases following surgery (Fremstad, Bergerud, Haffner & Lunde, 1976). The importance of this protein rests in its behavior as an acute phase reactant, the concentration of α_1 -acid glycoprotein increasing in various inflammatory states (Schmid, 1975). Such an increase in concentration has been shown to be accompanied by a decreased free fraction of propranolol (also a highly bound basic drug) in active Crohn's disease and inflammatory arthritis (Piafsky, Borga, Odar-Cederlof, Johnsson & Sjöqvist, 1978). The high affinity of prazosin for α_1 -acid glycoprotein demonstrated in this study suggests that similar changes might be seen in prazosin free fraction during inflammation.

Since propranolol and prazosin are frequently co-administered, and since both are basic drugs, the possibility of a protein binding displacement interaction was investigated. However, over a wide range of propranolol concentration there was no change in prazosin free fraction in human plasma, indicating that a clinically significant binding interaction between these two drugs is unlikely to occur. Most of the heart failure and renal failure patients were taking drugs which were not tested *in vitro* for binding interaction with prazosin. However, prazosin free fraction in patients with the same disease state did not show a trend corresponding to administration of any individual drug. Red blood cell to plasma partitioning studies revealed that 20% of prazosin is associated with erythrocytes in whole blood, and that equilibrium is very rapidly established between the red cell and plasma phases. Information on partitioning between red cells and plasma is useful in comparing prazosin concentrations measured in whole blood, as performed in this laboratory (Yee, Rubin & Meffin, 1979) with those measured in plasma. For drugs which are extensively bound to plasma proteins only a very small proportion of total drug in blood is in the unbound state. Since it is this free drug which is presumed to be in equilibrium with drug at the receptor site, small changes in the ability of proteins to accommodate such highly bound molecules can result in large percentage increases in the concentration of active drug, even though total drug concentrations might not change. This is specially relevant for drugs with small volumes of distribution, though this parameter is unknown for prazosin.

Using an ultrafiltration method, prazosin was reported to be 97% bound to human plasma proteins (Hobbs *et al.*, 1978). The high protein binding of prazosin has been confirmed in the present study with

a different methodology: [^{14}C]-prazosin of high specific activity has enabled binding to be assessed by equilibrium dialysis. In normal subjects, the free fraction of prazosin was 0.051 ± 0.007 (Table 1).

On the basis of this information, it would appear that the free fraction of prazosin could be substantially altered in disease states which result in quantitative or qualitative changes in plasma proteins. However, prazosin is a basic compound and it has been demonstrated that such drugs have different protein binding characteristics compared with the more extensively studied acidic drugs. Thus in chronic renal failure the binding of acidic drugs is commonly decreased while that of basic drugs is usually unchanged. This subject has recently been reviewed (Reidenberg, 1976). In cirrhosis, however, the protein binding of basic and acidic drugs appears to be affected to a similar extent (Blaschke, 1977) although one group has recently reported the binding of propranolol to be normal in this condition (Piafsky *et al.*, 1978). The mean free fraction of prazosin was significantly elevated both in cirrhotics and in patients with chronic renal failure (Table 1). In the cirrhotic group, mean prazosin free fraction was increased by 25% and in the chronic renal failure patients by 50%. The mean free fraction in patients with congestive heart failure was also increased, but the changes for the group as a whole failed to reach statistical significance. All three types of patients showed a considerably wider range of prazosin free fraction than that observed in normal subjects, and at the upper extremes this could exceed the highest normal free fraction by 100% or more (Table 1). It may be concluded, therefore, that in all three types of disease states studied the free fraction of prazosin can substantially exceed that in subjects with normal renal, hepatic and cardiac function. The clinical consequences of changes of this magnitude in the free fraction will depend on a number of other factors, such as redistribution, the shape of the concentration-response relationship in the therapeutic range, and whether hepatic clearance of prazosin is blood flow or capacity limited (Blaschke, 1977). Data on these variables are not available for prazosin at present.

From the practical stand point it would be helpful to predict which patients are likely to demonstrate impaired protein binding of prazosin. In the case of cirrhosis there was a highly significant linear correlation between prazosin free fraction and albumin concentration (Figure 2). This strongly implies that albumin plays an important role in the binding of prazosin. Of course, a good correlation does not necessarily imply cause and effect, and it could be argued that other biochemical changes occur at the same time as the fall in albumin concentration to explain this observation. However, this finding is in good agreement with the studies on prazosin binding to various concentrations of albumin in

solution where it was found that a decrease in albumin from 44 g/l to 22 g/l resulted in a 50% increase in prazosin free fraction. This is virtually identical to the increased free fraction seen over a similar range of albumin concentration in cirrhotics (Figure 2). The conclusion to be drawn from these findings appears to be that albumin is an important protein in the binding of prazosin. However, in both congestive heart failure and chronic renal failure patients, prazosin free fraction varied considerably but did not correlate with albumin concentrations. Indeed, the range of albumin concentration was small in both these groups (see **Methods**). Basic drugs have been reported to bind normally in uncomplicated chronic renal failure while acidic drugs usually bind abnormally but in a way which correlates poorly with albumin concentration (Reidenberg, 1976). This latter observation has been explained by the finding that endogenous nonpolar acidic compounds such as free fatty acids interfere with binding of acidic drugs to albumin (Dromgoole, 1973). Although prazosin is a base, it is perhaps a sufficiently weak base that such endogenous compounds interfere with its binding to albumin in chronic renal failure. However, prazosin free fraction correlated poorly with serum creatinine concentration while the binding of acidic drugs has been reported to correlate well with this parameter. The other possibility which clearly presents itself is that wide variations in α_1 -acid glycoprotein concentration are responsible for the increased range of prazosin free fraction observed in chronic renal failure. However, all these patients had renal impairment which was uncomplicated by inflammatory conditions and such subjects have been reported to have concentrations of α_1 -glycoprotein which are not statistically different from normal (Piafsky *et al.*, 1978).

Impaired protein binding in congestive heart failure has not been previously reported. In this study the mean free fraction of prazosin was not significantly different from normal but the range of free fraction was considerable in the heart failure patients, the upper extreme being 100% greater than the highest value in normals. The mechanism of this finding is unclear.

In conclusion, it has been demonstrated that substantial increases in free fraction of prazosin occur in cirrhosis, chronic renal failure and in certain patients with congestive heart failure. Caution should therefore be exercised in prescribing prazosin for patients with these conditions.

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