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The plasma protein binding of basic drugs

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Introduction

It should perhaps no longer surprise us that apparent setbacks in research often lead ultimately to important progress. In 1975, Cotham & Shand noted spuriously low plasma propranolol concentrations in blood samples collected in certain commercial collection tubes. This problem led to the discarding of data from several studies. A plasticiser (Tris-butoxy ethyl phosphate or TBEP) in the rubber stopper was found to displace the drug from its binding site in plasma, allowing it to enter red cells and resulting in the lower total plasma concentration (Piafsky & Borga, 1976). TBEP displaced drugs from a hitherto little studied protein, α_1-acid glycoprotein (AAG), which constituted only a small proportion of the total plasma proteins. Piafsky and colleagues subsequently showed that the protein binding of propranolol and chlorpromazine correlated closely with the concentration in the plasma of AAG in various diseases (Piafsky *et al.*, 1978).

Collection of plasma into similar tubes resulted in invalidation of 6 months of our own data on lignocaine, but led to the recognition of α_1-acid glycoprotein as a major determinant of the protein binding of this antiarrhythmic drug (Stargel *et al.*, 1979; Routledge *et al.*, 1980a). In the 10 years since Cotham and Shand's first observation, much has been learned about the plasma protein binding of other basic drugs (Piafsky, 1980), which had been largely ignored compared with acidic (anionic) compounds.

Binding to AAG

AAG is present in the plasma in concentrations which are normally 100 times lower than albumin. Its role in the body is unknown although it may play a part in normal coagulation, immunological and tissue repair processes (Schmid, 1975). It is a glycoprotein of molecular weight 40,000 and its high sialic acid content results in its acidic nature and low pKa. Binding of drugs to AAG appears to involve hydrophobic rather than electrostatic forces, since removal of sialic acid residues does not markedly reduce binding (van der Sluijs & Meijer, 1985). Some acidic drugs such as warfarin can compete with basic drugs for what appears to be a single binding site, perhaps in the protein part of the glycoprotein molecule (Urien *et al.*, 1982; Muller & Stillbauer, 1983). These acidic drugs appear to be chiefly the agents which bind at site I (e.g. warfarin) rather than site II drugs such as phenylbutazone. It is unlikely that AAG contributes significantly to the binding of such acidic drugs in plasma since the affinity and capacity of albumin for these drugs is so high.

Pharmacokinetic studies with radiolabelled AAG indicate that 60% of the protein is in the central compartment (probably the plasma) and the remainder in a peripheral compartment which is likely to be the extravascular space (Houin, personal communication). It is rare for basic drugs to bind solely to AAG although disopyramide and erythromycin may do so at therapeutic concentrations (Lima *et al.*, 1981; Barre *et al.*, 1984). Nevertheless, because of the binding characteristics of drugs to this protein, AAG is often the major determinant of variability in plasma protein binding both between individuals and within individuals. The plasma protein binding of basic drugs is variable partly because AAG concentrations may vary markedly in health and in disease and partly because the protein represents a high affinity, low capacity binding site and can be readily saturated by increasing drug concentrations. Some drugs for which AAG is a major determinant of plasma protein binding are listed in Table 1.

Binding to albumin

Albumin is the most important protein in the plasma and its concentration varies less than two-fold in health. It often contributes significantly towards the total binding of basic drugs in plasma. Fifty percent of the propranolol found in plasma is bound to albumin for example (Bel-

Table 1 Some basic drugs which bind significantly to α_1-acid glycoprotein (AAG)

β-adrenoceptor blockers
Alprenolol (Piafsky & Borga, 1977)
Oxprenolol (Belpaire *et al.*, 1982)
Pindolol (Belpaire *et al.*, 1982)
Propranolol (Piafsky *et al.*, 1978)
Timolol (Belpaire *et al.*, 1982)

Miscellaneous
Chlorpromazine (Piafsky *et al.*, 1978)
Dipyridamole (Kopitar & Weisenberger, 1971)
Erythromycin (Prandota *et al.*, 1980)
Metoclopramide (Webb *et al.*, 1986)
Nicardipine (Urien *et al.*, 1985)
Phencyclidine (Giles *et al.*, 1982)
Prednisolone (Milsap & Jusko, 1983)
Progesterone (Ganguly & Westphal, 1968)
Triazolam (Kobroth *et al.*, 1984)

Antiarrhythmics
Aprindine (Teirlynck *et al.*, 1982)
Bupivacaine (Denson *et al.*, 1984)
Disopyramide (Lima *et al.*, 1981)
Lignocaine (Routledge *et al.*, 1980a)
Pirmenol (Hammill *et al.*, 1982)
Quinidine (Nilsen *et al.*, 1978)
Verapamil (McGowan *et al.*, 1983)

Opiates
Methadone (Romach *et al.*, 1981)
Pethidine (Nation, 1981)

Antidepressants
Amitriptyline (Pike & Skuterud, 1982)
Imipramine (Borga *et al.*, 1977)
Nortriptyline (Pike & Skuterud, 1982)

paire *et al.*, 1982). Since albumin tends to show low affinity high capacity binding of basic drugs it is unusual for variation in albumin concentrations to result in marked changes in their plasma protein binding. Nevertheless, marked changes in albumin may result in changes in binding of basic drugs (Webb *et al.*, 1986). Hyperalbuminaemia is rare but hypoalbuminaemia can occur in severe liver or renal disease, particularly in the nephrotic syndrome. The treatment of chronic renal excretory failure with chronic ambulatory peritoneal dialysis (CAPD) results in marked albumin loss and hypoalbuminaemia as severe as that seen in the nephrotic syndrome (Leopold *et al.*, 1985). These changes may affect the plasma protein binding of some basic drugs depending on the contribution of albumin to the total binding of the drug in plasma.

Binding to other proteins

Lipoproteins, have been described to bind some basic drugs such as amitriptyline and nortriptyline (Pike *et al.*, 1983). Complement C3 has been reported to be related to imipramine binding (Kristensen, 1983) and the plasma protein binding of new non-steroidal anti-inflammatory agent, timegadine, was increased by enrichment of plasma with C reactive protein, α_1-antitrypsin and AAG (George *et al.*, 1984). The clinical relevance of these findings is unclear but it is unlikely that these proteins, which are present only in small amounts, are major determinants of the plasma protein binding of basic drugs.

Variability in protein binding

The protein binding of drugs which bind to AAG varies because of the wide variation in AAG concentrations in health and disease (Routledge *et al.*, 1982).

Pre-menopausal females appear to have slightly lower protein binding of lignocaine than males and this is probably because circulating oestrogens appear to reduce AAG concentrations (Song *et al.*, 1970; Routledge *et al.*, 1981b). Indeed exogenous oestrogens in the form of the oral contraceptive pill or given as therapy for carcinoma of the prostate may markedly reduce AAG (Routledge *et al.*, 1981b). The elderly have a slight increase in AAG concentrations but this is unlikely to result in marked changes of binding of basic drugs unless other diseases are present (Davis *et al.*, 1980; Paxton & Briant, 1984). AAG concentrations are low in the newborn and this may lead to marked reductions in plasma protein binding of basic drugs (Piafsky & Mpamugo, 1981; Wood & Wood, 1981).

Concentrations of AAG in plasma in spouses are more closely correlated than those between parent and child suggesting an important influence of shared environmental factors (Blain *et al.*, 1985). This suggestion is supported by the fact that the concentration of the protein in identical twins correlates very poorly (Storiko, 1968). Healthy subjects may have some variability in the concentration of AAG from day to day and even within the day (Yost & DeVane, 1985). However, large increases in AAG concentration can be related to intercurrent infection

and sometimes to concomitant drug therapy. For example, epileptic patients on anticonvulsant therapy (Routledge *et al.*, 1981a; Tiula & Neuvenon, 1982) and subjects receiving the tricyclic drug amitriptyline (Baumann *et al.*, 1982) appear to have higher AAG concentrations.

Disease-related changes in AAG are also extremely important. The concentration of AAG is increased in patients with chronic renal failure (Grossman *et al.*, 1982) but is reduced in those with the nephrotic syndrome (probably because of loss of the protein in the urine) and in hepatic cirrhosis (Barre *et al.*, 1984). AAG concentrations are also increased in obesity (Benedek *et al.*, 1983), trauma (Edwards *et al.*, 1982), burns (Martyn *et al.*, 1984), cancer (Jackson *et al.*, 1982), various inflammatory diseases such as rheumatoid arthritis (DeLeve & Piafsky, 1981), after surgery (Farndon *et al.*, 1982) and after myocardial infarction (Routledge *et al.*, 1980b, 1980c). The time course of these changes is relatively slow with the rise not being apparent until 24 h or later and reaching a peak at around 8–10 days and remaining elevated for as long as 3 weeks after myocardial infarction (Bachmann *et al.*, 1968).

In the first paper describing the relationship between AAG and the plasma protein binding of propranolol and chlorpromazine, Piafsky and co-workers (1978) reported a negative linear correlation between the proportion of drug free in plasma (free fraction) and AAG concentration. Although the relationship was regarded as linear, examination of the figure in their paper indicates a curvilinear relationship which becomes clearer when studies of lignocaine binding in diseases associated with a larger range of AAG concentrations are grouped together (Figure 1). The shape of the relationship is such that a given decrement from the mean AAG concentration will result in a proportionately greater change in free fraction of drug than the same increment from the mean. Thus diseases associated with marked reductions in AAG concentration are likely to be associated with greater changes in protein binding than diseases where the AAG is increased. The relationship can be made linear by expressing the binding as the ratio of bound (B) to free (F) drug concentration.

$$\frac{B}{F} = \frac{nP}{K_d + F} \qquad I$$

where n is the number of binding sites per protein molecule, P is the molar concentration of binding protein and K_d is the dissociation constant of the drug-protein complex. When K_d is large compared with the free drug concentration (F), a linear relationship between the binding ratio and AAG concentration in plasma will be seen (Nilsen *et al.*, 1978). A similar relationship will be seen with binding and the concentration of other proteins but since albumin is the only other major binding protein and its affinity is generally low, it can normally be ignored: thus for many basic drugs the binding is linearly related to AAG concentration. For drugs which bind mainly to AAG, changes in non-esterified fatty acid concentrations do not appear to result in altered protein binding, in contrast to drugs in which albumin is the major binding protein (Grossman *et al.*, 1982). Endogenous inhibitors of binding to AAG do not appear to be important in chronic renal failure when AAG is the major binding protein (Grossman *et al.*, 1982). However, when albumin is an important determinant of the total binding, there may be a decrease in binding in chronic renal failure greater than would be expected from the changes in AAG and albumin alone (Webb *et al.*, 1986).

Protein binding and pharmacokinetics

Changes in protein binding of basic drugs will have marked effects on distribution and clearance of the drug. Seventy percent of AAG is in the intravascular space and only 30% lies extravascularly. It is, therefore, likely that increased AAG concentrations will result in reduced volumes of distribution of the drug. Increases in AAG concentration will also affect the distribution of drug between plasma and red cells which often act as a reservoir for the drug in blood. The effects are complex but there is a curvilinear relationship between AAG concentration and the red cell to plasma ratio of lignocaine (Routledge *et al.*, 1981a).

Finally, changes in protein binding of basic drugs will result in changes in clearance. Using the well-stirred vessel model (Wilkinson & Shand, 1975), clearance (CL) can be expressed by the following equation:

$$CL = Q\left(\frac{CL_i.f_u}{Q + CL_i.f_u}\right) \qquad II$$

where CL_i is the intrinsic clearance of the drug by the organ of elimination, f_u is the fraction of drug unbound in blood and Q is the blood flow to the eliminating organ (normally the liver). When the expression $CL_i.f_u$ is small compared with the blood flow (Q) the equation simplifies to:

$$CL = CL_i.f_u \qquad III$$

and total drug clearance is directly proportional to the fraction unbound in blood. Drugs which

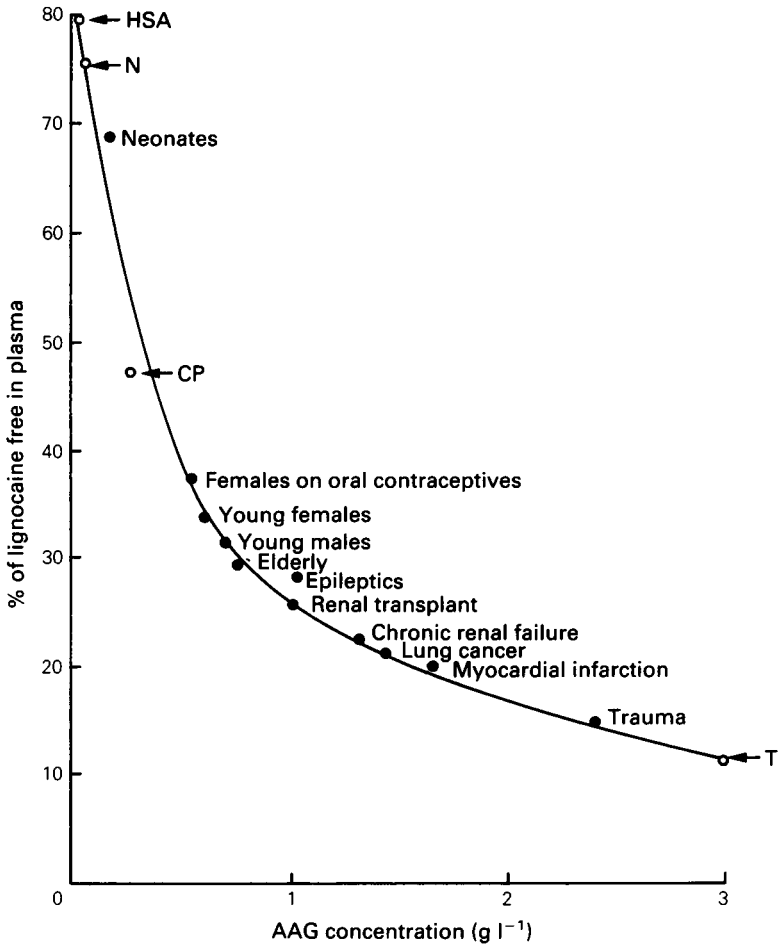

Figure 1 Relationship between mean α_1-acid glycoprotein (AAG) concentration and the mean percentage of lignocaine free in plasma. For references see text. Open symbols indicate single individuals. T = trauma (Edwards *et al.*, 1982), CP = carcinoma of the prostate on oestrogens (Routledge *et al.*, 1981b), N = neonate (Wood & Wood, 1981), HSA = human serum albumin 40 g l^{-1} (Routledge *et al.*, 1980a).

follow this pattern have been called 'low clearance' or 'restrictively eliminated' drugs. An increase in protein binding would result in reduced total clearance of the drug but the effect at steady state would be unchanged since the average free drug concentration would remain constant.

Most basic drugs, however, behave in a different fashion. When CL_i is much larger than Q, Equation II simplifies to:

$$CL = Q \qquad IV$$

If the liver is the major eliminating organ, clearance will approach the value of liver blood flow (approximately 1.5 l min^{-1}) and will be independent of changes in f_u. Thus the intravenous total plasma clearance of propranolol appears to be independent of the binding in blood. Free drug clearance will change, however, since the amount of drug in the bound form has changed. After intravenous administration of (+)-propranolol (which does not affect liver blood flow) clearance was not significantly correlated with f_u ($r = 0.3$) but the area under the curve for free drug (AUC free) was ($r = 0.74$) (DeLeve & Piafsky, 1981). Since (+)-propranolol has little pharmacological activity the effects of the changes in AUC free on drug activity could not be measured and no other relevant studies have been reported in man. Nevertheless, recent studies in the pithed rat in which inflammation had been experimentally

induced by turpentine oil have shown a decreased β-adrenoceptor blocking effect of intravenous administered oxprenolol and propranolol compared with controls (Mugabo *et al.*, 1984a). The effect of two other β-adrenoceptor blockers which do not significantly bind to AAG (metoprolol and atenolol) was not changed, and these findings support the theoretical considerations described above. Further supportive evidence has been published by the same group using the same model of experimentally induced inflammation and intravenous infusion of isoprenaline to induce vasodilation in perfused rat hind quarters before and after propranolol or sotalol administration (Mugabo *et al.*, 1984b).

For low clearance drugs, the effects of protein binding changes will be independent of the route of drug administration but for high clearance drugs, these considerations apply only to systemic administration. High clearance drugs undergo substantial pre-systemic elimination after the oral route and it has been suggested that increased protein binding will reduce pre-systemic elimination. The resultant increased bioavailability will then offset the effect of the reduced f_u on systemic clearance of these agents and the free drug concentration will be unchanged. Although no examples of this phenomenon have been demonstrated in man, intraportal (analogous to oral) administration of propranolol and metoprolol was not associated with changes in β-adrenoceptor blocking effect in the rat model in which inflammation and therefore increased drug binding had been experimentally induced, unlike the situation previously described for intravenous drug (Mugabo *et al.*, 1984a). Similar results were obtained in the perfused rat hindquarters, indicating that the effect of increased binding on the response to drugs bound to AAG may indeed depend on the route of administration (Mugabo *et al.*, 1984b).

Unfortunately, the situation in man is often more complicated. AAG (and therefore drug binding) may be continually changing with time so that a steady-state never occurs. The AAG concentration in blood (and therefore the protein binding of lignocaine) continues to rise for several days after myocardial infarction. When lignocaine is infused intravenously, total plasma and blood lignocaine concentrations continue to rise when steady state should have been achieved (Figure 2), although the free plasma lignocaine concentration (and presumably drug effect) rises less. Such changes may be related in part to changes in tissue distribution and to reduced free clearance of this 'high clearance' drug caused by

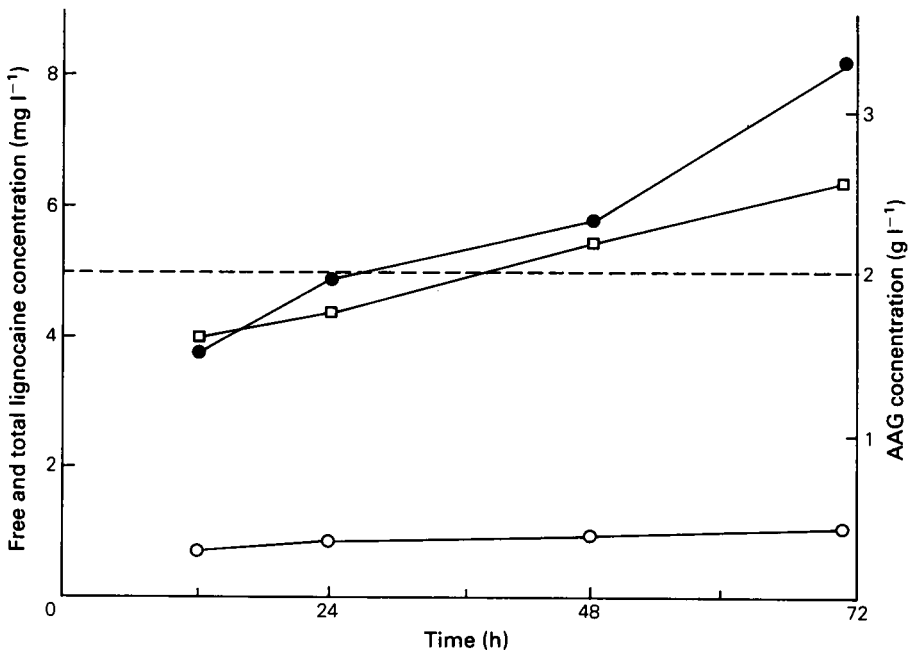

Figure 2 Total (●) and free (○) plasma lignocaine concentrations and AAG (□) concentration in a 54 year old male given lignocaine (2 mg min^{-1}) by intravenous infusion for 72 h after myocardial infarction. No symptoms or signs of lignocaine toxicity occurred at any time during the infusion.

an increase in plasma protein binding. Nevertheless, the free plasma lignocaine concentration does increase slightly (although to a much smaller extent than total plasma and whole blood concentrations) (Barchowsky *et al.*, 1982) and this has been explained by a time-dependent fall in intrinsic free clearance of drug which has been seen in normal volunteers after prolonged lignocaine administration (Ochs *et al.*, 1980). More than one phenomenon, therefore, appears to be occurring in this situation.

Protein binding and drug effects

The clinical consequences of changes in protein binding will most often be seen if the total plasma drug concentration is used as an index of efficacy or toxicity. Whereas total plasma lignocaine concentrations greater than 5 mg l^{-1} are usually associated with toxicity, patients after myocardial infarction may have total plasma concentrations as great as 8 mg l^{-1} without showing any signs of toxicity (Figure 2). This is because the increase in AAG concentration results in a greater proportion of the total plasma concentration being in the bound and therefore inactive form. The toxicity of lignocaine appears to be related more closely to free than to total plasma lignocaine concentration (Pieper *et al.*, 1980) just as the β-adrenoceptor blocking efficacy of propranolol is related to free than total plasma propranolol concentrations (McDevitt *et al.*, 1976).

It is clear from these observations that measurement of free drug concentration would be more useful clinically than the measurement of total plasma concentration. Unfortunately no rapid and reliable method to measure free drug concentration is presently available for basic drugs. This may be because basic drugs seem to bind non specifically to materials such as membranes (and sometimes even glass) and ultrafiltration devices appear to give variable results. Equilibrium dialysis techniques are often reliable, but results can usually only be obtained after 2 or more hours. Indirect measures of assessing free drug concentrations also have drawbacks. Salivary concentrations are unlikely to be of value since the pK of these drugs is often close the pH of saliva and changes in the pH of saliva will result in marked interindividual differences in the partition of the drug between saliva and blood (Mucklow, 1982). If the binding of the drug is closely related to protein concentration, it is possible to measure the protein concentration rapidly and reliably using nephelometric means. By measuring AAG concentration together with the total drug concentration, it is possible to estimate the free plasma lignocaine concentration although rapid determination of protein concentration requires relatively expensive apparatus (Routledge *et al.*, 1982, 1985). Thus none of the indirect techniques are ideal and the search must continue for rapid and reliable direct measures of free drug concentration.

Summary

The plasma protein binding of basic drugs appears to vary more than was at first assumed and is related to the marked intra-and interindividual differences in one of the chief binding proteins, AAG. Changes in AAG concentrations will result in alterations in the distribution and metabolism of basic drugs which will complicate the interpretation of the relationship between total drug concentration and drug efficacy or toxicity. For some drugs, e.g. lignocaine, direct measurement of free concentrations may improve their clinical use but rapid and reliable techniques are as yet not readily available.

I acknowledge the major contribution made by the late Ken Piafsky to the study of protein binding of basic drugs, and I thank David Shand for encouragement and advice during our own work on this subject.

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