COMMENTARY

Changes in plasma protein binding have little clinical relevance

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Although a number of articles have appeared in the literature that question the clinical importance of changes in plasma protein binding,[1-5] many clinicians, regulators, industrial drug developers, and health science academicians are still concerned about and want to test for the potential clinical relevance of drug-drug interactions and disease-drug interactions that lead to increased free fractions of drugs in the plasma or blood. This concern is based on the intuitive belief that when a drug is displaced from its plasma binding protein, increased unbound drug concentrations will cause an increase in drug effect and potential toxic

results. Rolan,[3] Holford and Benet,[5] and particularly Sansom and Evans[4] have presented theoretic arguments about the limited cases when protein-binding changes could be clinically significant, but the message appears not to have been heard. We now address the issue using a systematic approach of exposure and equilibration time concepts that will allow concerned clinicians, academicians, industrial scientists, and regulators to more fully understand the very limited cases when protein-binding changes may be important clinically.

What is the basis for the idea that protein-binding interactions lead to clinically significant changes in drug effects? Three reports of studies in humans in the mid-1960s are probably the source. In 1963 Christensen et al[6] reported sulfaphenazole-induced hypoglycemia in tolbutamide-treated diabetic subjects, whereas in 1964 Fox[7] described the potentiation of anticoagulants by pyrazole compounds.

This enhanced anticoagulant effect was further described in a very influential article by Aggeler et al.[8] Those investigators showed that warfarin administered with phenylbutazone not only increased plasma levels of warfarin but also significantly increased prothrombin times in normal volunteers. In an attempt to explain these observations, Aggeler et al[8] examined the protein

binding of warfarin alone and in the presence of phenylbutazone. In this in vitro experiment, they clearly showed that phenylbutazone displaced warfarin from its albumin binding sites. From these two experiments, an in vivo drug interaction observation and an in vitro protein-binding experiment, they proposed a cause for a clinical observation; that is, an increase in fraction unbound in plasma (f_u) caused the changes in pro-thrombin times by increasing the concentration of unbound drug. The problem is in the extrapolation from an in vitro observation to an in vivo effect.

In reality, the clinical interactions observed with the anticoagulant and antidiabetic drugs described, as well as a number of others reviewed by MacKichan,[2] Rolan,[3] and Sansom and Evans,[4] result from changes in drug metabolic clearance and not from changes in protein binding. However, because the protein-binding changes do cause changes in pharmacokinetic parameters in certain cases, the belief in the clinical importance of these changes has persisted. We first review the basis for the pharmacokinetic parameter changes.

PHARMACOKINETIC PARAMETERS

There is clear evidence that plasma protein binding is relevant in the pharmacokinetic modeling of drugs, as has been primarily emphasized in terms of individual pharmacokinetic parameters[9]; that is, the volume of distribution (V)

$$V = [f_u/f_{uT}] V_T + V_P \qquad (1)$$

depends on the fraction unbound in plasma (f_u), the fraction unbound in tissue (f_{uT}), the volume of tissue (V_T), and the volume of plasma (V_P).[10] For all drugs with a V value $\geq$30 L (when V_P has only a minor effect on V), changes in f_u therefore translate directly into changes in V.[10]

All organ clearance models (here we use the simplest well-stirred venous equilibrium model[10,11]) incorporate a protein-binding term

$$CL = [Q_{organ} \cdot f_u \cdot CL_{int}]/[Q_{organ} + f_u \cdot CL_{int}] \qquad (2)$$

in which CL is organ clearance, Q_{organ} is blood flow to the clearing (eliminating) organ, and CL_{int} is the intrinsic organ clearance of the unbound drug. High extraction ratio drugs ($Q_{organ} << f_u \cdot CL_{int}$) exhibit organ clearance independent of f_u (ie, $CL \cong Q_{organ}$), but for low extraction ratio drugs ($Q_{organ} >> f_u \cdot CL_{int}$)

$$CL \cong f_u CL_{int} \qquad (3)$$

clearance depends on f_u and the intrinsic ability of the organ to clear the drug (CL_{int}).[9,10]

Again, with use of the well-stirred model as an example, the hepatic bioavailability (F_H) is given by the following:

$$F_H = Q_H/[Q_H + f_u \cdot CL_{int}] \qquad (4)$$

Then $F \cong 1$ for a low extraction ratio drug, but for a high extraction ratio drug

$$F_H \cong Q_H/[f_u \cdot CL_{int}] \qquad (5)$$

in which F_H is inversely related to f_u and CL_{int} and is also directly dependent on Q_H.

Because the half-life ($t_{1/2}$) may be defined in terms of the ratio of volume to clearance multiplied by ln2, it is recognized that for high extraction ratio drugs, when V $\geq$30 L, this parameter will also depend on f_u, as follows:

$$t_{1/2} \cong [0.693(f_u/f_{uT})V_T]/Q_{organ} \qquad (6)$$

However, $t_{1/2}$ is independent of f_u for low extraction ratio drugs, for which V $\geq$30 L, as follows:

$$t_{1/2} \cong [0.693(V_T/f_{uT})]/CL_{int} \qquad (7)$$

It is therefore correct that, depending on the pharmacokinetic parameters measured and the intrinsic clearance of the drug, certain pharmacokinetic parameters will change with protein binding but others will not. Furthermore, the changes in the individual pharmacokinetic parameters may result in changes in the observed concentration–time profiles.[12] However, the belief that the effective concentration of all drugs depends on protein binding is not correct, as we will show in the next section.

EXPOSURE CONCEPTS

The introduction of clearance concepts in the mid-1970s has had a major impact on recognizing the relevance of pharmacokinetics to the clinical practice of medicine. However, the pendulum has swung too far, and we now overemphasize the effect of disease states and drug interactions on individual pharmacokinetic parameters rather than on the most relevant measure, drug exposure.

Exposure is a term that reflects the drug levels to which a patient is exposed after a dose or a series of doses. It is a measure of concentration integrated over time, commonly referred to as area under the curve (AUC). In some cases, particularly for toxicity issues, the clinician may be concerned about the maximum exposure of drug at a particular time (C_{max}) or that systemic concentrations be maintained above a threshold minimum effective concentration. However, we will consider the integrated exposure, AUC, because it is the parameter directly related to dose, as follows:

$$AUC = [F \cdot Dose]/CL \qquad (8)$$

First, let us look at oral dosing. When we dose orally, bioavailability (F_{oral}) is the product of three availability factors (assuming negligible lung first-pass effects) as follows:

$$F_{oral} = F_{abs} \cdot F_G \cdot F_H \qquad (9)$$

in which F_{abs} is the fraction of administered drug that is absorbed into the gut wall and does not flow back into the lumen, F_G is the fraction that gets through the gut wall unchanged, and F_H is that fraction that passes through the liver and into the systemic circulation unchanged.[13] Therefore, when a drug that is eliminated primarily by the liver is given orally, systemic AUC can be calculated by inserting equations 2, 4, and 9 into equation 8 to give the following:

$$AUC_{oral} = [F_{abs} \cdot F_G \cdot Dose]/[f_u \cdot CL_{int}] \qquad (10)$$

Equation 10 is general and holds for both high and low extraction ratio drugs that are cleared by the liver and given orally. It is the general consensus that pharmacologic effect is related to exposure to unbound drug concentrations (AUC^u). Therefore, for oral dosing when systemic elimination occurs from the liver, AUC^u will be given by the following equation:

$$AUC^u_{oral} = f_u \cdot AUC_{oral} = [F_{abs} \cdot F_G \cdot Dose]/CL_{int} \quad (11)$$

Note that in equation 11 changes in f_u have no effect on unbound drug exposure; therefore no changes in pharmacologic effect would be expected for drugs that are administered orally and eliminated hepatically (Table I).

For any drug given orally when systemic elimination is not hepatic ($F_H = 1$) or for any drug given intravenously ($F = 1$), then substituting the definition of organ clearance (equation 2) into equation 8 yields the following:

$$AUC^u_{oral,nonhepatic} = AUC_{IV} = [F_{abs} \cdot F_G \cdot Dose$$
$$(Q_H + f_u \cdot CL_{int})]/[Q_H \cdot f_u \cdot CL_{int}] \qquad (12)$$

in which F_{abs} and F_G for intravenous dosing equal 1.

For a low extraction ratio drug ($Q_{organ} \gg f_u \cdot CL_{int}$) after intravenous dosing

$$AUC^u_{IV} = f_u \cdot AUC_{IV} \cong Dose/CL_{int} \qquad (13)$$

and changes in f_u will not affect unbound exposure. This will also be the case for low extraction ratio drugs cleared nonhepatically when they are administered orally, although F_{abs} and F_G will need to be considered, as follows:

$$AUC^u_{oral,nonhepatic} \cong [F_{abs} \cdot F_G \cdot Dose]/CL_{int} \qquad (14)$$

Table I. Summary of types of drugs and routes of administration for which protein-binding changes may be clinically relevant

	High extraction ratio	*Low extraction ratio*
Intravenous administration		
Hepatic clearance	Yes*	No
Nonhepatic clearance	Yes*	No
Oral administration		
Hepatic clearance	No	No
Nonhepatic clearance	Yes†	No

*See Table II for drugs that meet these criteria.
†No drugs from a list of 456 drugs[14,15] met these criteria.

In summary, for all low extraction ratio drugs, regardless of route of administration, and for all drugs administered orally and eliminated primarily by the liver, total exposure is independent of protein binding and no dosing adjustments will need to be made for real or anticipated changes in f_u (Table I). Only high extraction ratio drugs given intravenously and oral drugs eliminated by nonhepatic high extraction ratio routes will exhibit changes in unbound drug exposure when protein binding changes; that is, for a high extraction ratio drug ($Q_{organ} \ll f_u \cdot CL_{int}$), equation 12 becomes the following:

$$AUC_{IV} \cong Dose/Q_H \qquad (15)$$

and unbound AUC is obtained by multiplying both sides of equation 15 by f_u, as follows:

$$AUC^u_{IV} = f_u \cdot AUC_{IV} \cong [f_u \cdot Dose]/Q_H \qquad (16)$$

This will also be the case for high extraction ratio drugs not cleared by the liver when they are administered orally, once F_{abs} and F_G are considered as factors.

APPLICATION OF PRINCIPLES

We can now explain why changes in protein binding cannot be important for warfarin. Warfarin is eliminated by hepatic metabolism but is a low extraction ratio drug, so $F_H \cong 1$ and in fact $F_{oral} = 1$.[14,15] Therefore its total systemic exposure is described by equation 10 and its unbound exposure by equation 11, which is independent of protein binding. Changes in f_u caused by either disease effects or drug interactions will therefore not be expected to influence clinical outcome, and no adjustment of drug dosing should be required.[3,4] The effect of phenylbutazone on warfarin levels and efficacy can be explained by noting that phenylbutazone inhibits warfarin metabolism (CL_{int} decreases).[16] In

Table II. The 25 drugs in a list of 456 drugs[14,15] for which protein binding may influence clinical drug exposure after nonoral administration, with use of cutoffs of >70% for protein binding ($f_u < 0.3$) and ≥ 0.28 Q_{organ} for clearance

	Protein binding (%)	CL (ml/min · kg)
Alfentanil*	92	10.6§
Amitriptyline†‡	95	11.5§
Buprenorphine*†	96	13.3§
Butorphanol*†‖	80	22§
Chlorpromazine*‡	95	8.6§
Cocaine*‖	91	32§
Diltiazem*‡	78	11.4§
Diphenhydramine*‡	78	6.2§
Doxorubicin*	76	16.2§
Erythromycin*‡	84	8.0§
Fentanyl*	84	12.3§
Gold sodium thiomalate (INN, sodium aurothiomalate)†	95	4.8¶
Haloperidol†‡	92	11.8§
Idarubicin*‡	97	29§
Itraconazole*‡	99.8	12.7§
Lidocaine*	70	9.2§
Methylprednisolone*†‡	78	6.2§
Midazolam*†‡	98	6.6§
Milrinone*	70	5.2¶
Nicardipine*‡	99	10.4§
Pentamidine*	70	16§
Propofol*	98	27§
Propranolol*‡	87	18§
Remifentanil*	92	40-60#
Sufentanil*	93	12§
Verapamil*‡	90	15§

f_u, Fraction unbound in plasma; Q_{organ}, blood flow to the clearing (eliminating) organ.
*Intravenous administration.
†Intramuscular administration.
‡Does not apply for oral administration of this drug.
§>0.28 Q_H; $CL_H \geq 6$ ml/min per kilogram; see text for explanation for this low cutoff for "high."
‖Nasal delivery.
¶>0.28 Q_R; $CL_R \geq 4.8$ ml/min per kilogram; see text for explanation for this low cutoff for "high."
#Probably metabolized in blood by nonspecific esterases.

fact, the package insert for warfarin does not recommend a change in dose with real or anticipated changes in f_u. This recommendation is based on clinical experience rather than on pharmacokinetic principles, but we have just shown that it is solidly grounded in the analysis of drug exposure.

There are, of course, as we have shown, situations in which f_u becomes a determinant of AUC^u. The first is high extraction ratio drugs that are eliminated primarily by hepatic metabolism when they are administered intravenously (equation 16). The second case is high extraction ratio drugs given either orally or intravenously when the liver is not the main route of systemic elimination, as follows:

$$AUC^u_{IV} = [f_u \cdot Dose_{IV}]/Q_R \qquad (17)$$

or

$$AUC^u_{oral} = [F_{abs} \cdot F_G \cdot f_u \cdot Dose_{oral}]/Q_R \qquad (18)$$

in which we have assumed renal elimination and used renal blood flow (Q_R) in the organ clearance equation (equation 2).

An examination of 456 drugs[14,15] revealed that none which are administered orally come close to meeting the criteria of nonhepatic elimination (>50% excreted unchanged), significant protein binding (>70% bound to plasma proteins), or high nonhepatic extraction ratio clearance (>0.5 Q_R; >8.5 ml/min per kilogram). They do not meet the criteria even when the high nonhepatic extraction ratio clearance cutoff is lowered to >0.28 Q_R, or >4.8 ml/min per kilogram. However, there are drugs that are administered by nonoral routes which meet the criteria of having significant protein binding (>70%) and having either a high hepatic or a high nonhepatic

Table III. Drugs for which changes in protein binding have been thought to be important and reasons these changes are not clinically relevant

Drug	Reason
Carbamazepine	Only given orally; low hepatic extraction ratio (0.08)
Ceftriaxone	Low hepatic extraction ratio (0.01)
Chlorpropamide	Very low hepatic extraction ratio (0.001)
Diazepam	Low hepatic extraction ratio (0.02)
Ketoprofen	Only given orally; low hepatic extraction ratio (0.06); probably long equilibration time
Methotrexate	Low protein binding (46%); low hepatic extraction ratio (0.06); probably long equilibration time
Phenytoin	Low extraction ratio (~0.03 in linear range, decreases with higher saturation concentrations)
Tolbutamide	Only given orally; low hepatic extraction ratio (0.01); long equilibration time
Valproic acid	Very low hepatic extraction ratio (0.005)
Warfarin	Only given orally; very low hepatic extraction ratio (0.002); long equilibration time

extraction ratio clearance (Table II). To be as inclusive as possible, in Table II we list therapeutic agents that have protein binding of ≥70% and an extraction ratio of ≥0.28[14,15]; that is, hepatic clearance ≥6.0 ml/min per kilogram or renal clearance ≥4.8 ml/min per kilogram. We expanded the extraction ratio criteria far beyond those usually considered to be "high." We did this in part because the correct extraction ratio calculation should be organ blood clearance divided by organ blood flow, whereas plasma clearances are given in Table II for all of the drugs except amitriptyline and propranolol.[14,15] In Table II we included drugs that are given intravenously, intramuscularly, or intranasally. However, drugs administered intramuscularly or intranasally may be incompletely absorbed from the site of administration, and therefore the equations for AUC (equation 15) and AUC^u (equation 16) will need to be adjusted for that possibility.

THERAPEUTIC INDEX AND KINETIC-DYNAMIC EQUILIBRATION TIME

The listing in Table II suggests that only 25 drugs have the potential for exhibiting changes in clinical response, with changes in protein binding caused either by drug interactions or by disease states. As stated, a number of these are definitely borderline cases. Furthermore, this list would be even shorter if we were to consider the therapeutic index of each drug because if a drug has a wide therapeutic index (eg, propranolol), changes in free drug concentrations that result from protein-binding changes will have negligible clinical effects.

However, there is another pharmacokinetic-pharmacodynamic parameter that can expand the list of drugs beyond those in Table II. This parameter, developed by Sheiner et al[17] and Holford and Sheiner,[18] describes the delay between drug effects and drug concentrations in terms of a pharmacokinetic-pharmacodynamic equilibration half-time. Changes in protein binding caused by a drug interaction are assumed to instantaneously change free drug concentrations. Thus there should be a transient change in free concentrations while the body re-equilibrates. Drug distribution and drug elimination will change to compensate for the increased free drug clearance. When f_u increases, the displaced drug will distribute throughout the volume of distribution and the elimination rate will increase (if CL is unchanged). After about 4 distribution half-lives, the unbound concentration will return to its previous steady-state level even if the displacer continues to be present. If a drug has a very short pharmacokinetic-pharmacodynamic equilibration half-time (ie, drug effects appear to be directly related to free drug plasma concentrations), then an enhanced pharmacodynamic response could occur during the brief time in which the free concentrations are elevated. Drugs that exhibit such a short pharmacokinetic-pharmacodynamic equilibration half-time include antiarrhythmic agents, anesthetic agents, and pain medications, particularly those subject to abuse. (As far as we know, no other general compilation exists of pharmacokinetic-pharmacodynamic equilibration half-times beyond the initial presentation.[18]) Of the drugs listed in Table II, lidocaine is an example of a therapeutic agent that has a narrow therapeutic index and a very rapid equilibration time. Propafenone (protein binding, 85%-90%; hepatic plasma clearance, 17 ml/min per kilogram) is not listed in Table II because it is not available as an intravenous dose. However, because this highly protein-bound, high hepatic clearance drug has a short equilibration time and a narrow therapeutic index, changes in protein binding, even after oral administration, have the potential to yield a clinically meaningful response during the time that it takes for the body to re-equilibrate (proba-

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