Free drug fraction vs. free drug concentration: a matter of frequent confusion

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'In plasma, alteration of drug binding results in a decrease (or an increase) in the unbound fraction of a drug and thus in a reduced (or increased) effect.'

This classical sentence encountered in numerous articles on veterinary pharmacology is almost invariably wrong. It stems from a major confusion between two variables: the free fraction of a drug in plasma (fu) and its free plasma concentration (C_{free}). For most drugs, effects are governed by free drug concentrations and therefore can be altered by factors which modify the free drug *concentration*, which is not equivalent to changing the free drug *fraction,* as explained in this note.

PRELIMINARY DEFINITIONS

By definition fu is given by the relation

$$fu = C_{free}/C_{tot} \quad (1)$$

where C_{tot} is the total drug plasma concentration, generally measured by an analytical technique, and fu and C_{free} are as defined above.

The confusion between fu and C_{free} arises from the rearrangement of eqn 1 into eqn 2

$$C_{free} = fu \times C_{tot} \quad (2)$$

Equation 2 is appropriately used to compute C_{free} from an experimentally measured C_{tot} and a known fu, but use of this equation for mechanistic purposes, suggesting that a modification of fu is automatically responsible for a modification of C_{free}, is incorrect in the *in vivo* situation.

On the contrary, for most drugs an increase (or decrease) in fu corresponds *in vivo* to a decrease (or increase) in C_{tot}, with no alteration of C_{free}.

FACTORS CONTROLLING fu

By definition, C_{tot} is given by the relationship

$$C_{tot} = C_{free} + C_{bound} \quad (3)$$

where C_{bound} is the bound concentration. For a drug having a single family of binding sites (binding sites characterized by the same affinity constant K_A), C_{bound} is given by the general equation:

$$C_{bound} = (B_{max} \times C_{free})/(K_D + C_{free}) \quad (4)$$

where B_{max} is the maximal binding capacity (related to the molar concentration of the binding protein) and K_D is the equilibrium dissociation constant (equal to the inverse of the affinity constant K_A).

Incorporating eqn 4 into eqn 3 gives

$$C_{tot} = C_{free} + (B_{max} \times C_{free})/(K_D + C_{free}) \quad (5)$$

and after factorization, eqn 5 becomes

$$C_{tot} = [1 + (B_{max})/(K_D + C_{free})] \times C_{free} \quad (6)$$

From eqns 1 and 6 it can be shown that fu is given by

$$fu = (K_D + C_{free})/(B_{max} + K_D + C_{free}) \quad (7)$$

Inspection of eqn 7 shows that fu is expressed as a function of the free drug concentration C_{free} and the two binding parameters B_{max} and K_D.

Case of linear binding

In vivo, for many drugs the range of therapeutic free concentrations is much lower than K_D ($K_D >> C_{free}$) and eqn 7 can be simplified as:

$$fu = K_D/(B_{max} + K_D) \quad (8)$$

In this situation, which corresponds to drugs exhibiting linear binding, fu is independent of C_{free} and the drug plasma binding is therefore characterized by a constant unbound fraction (fu) over the range of concentrations encountered *in vivo*. Thus, in the case of drugs with linear plasma binding, fu can be increased or decreased by modifications of K_D and/or B_{max}. In contrast, in the nonlinear case (eqn 7), C_{free} cannot be ignored with respect to K_D and fu becomes a variable influenced by C_{free} (Toutain *et al.*, 2000b). This situation occurs infrequently and will not be considered further in the following discussion.

FACTORS CONTROLLING DRUG CONCENTRATIONS *IN VIVO*

The two major determinants of the plasma concentrations of either endogenous compounds or xenobiotics are the input and output rates of the analyte into plasma (Toutain *et al.*, 2000a). By definition, the rate of elimination of a drug from the body is given at any time by the following:

$$\text{Rate of elimination} = Cl_{tot} \times C_{tot} = Cl_{free} \times C_{free} \quad (9)$$

where Cl_{tot} and Cl_{free} are the clearances corresponding to the total and free drug in plasma, respectively. During repeated drug administration, steady state plasma concentrations are achieved when the rate of drug elimination becomes equal to the entry rate (ER) in the systemic circulation:

$$\text{Rate of elimination} = \text{ER} \quad (10)$$

It can be deduced from eqns 9 and 10 that the steady state plasma concentration (C_{ss}) is determined only by the ER and the drug clearance (Cl):

$$C_{ss} = \text{ER}/Cl \quad (11)$$

Entry rate is equal to the infusion rate (K_0) in the case of intravenous infusion and to $(F \times \text{Dose})/\tau$ in the case of multiple dosing, where F is the systemic bioavailability and τ the dosing interval (recalling that for multiple dosing, C_{ss} corresponds to the average concentration over τ).

The total ($C_{ss,tot}$) and free ($C_{ss,free}$) steady state drug concentrations are determined by the total and free clearances:

$$C_{ss,tot} = \text{ER}/Cl_{tot} \quad (12)$$

$$C_{ss,free} = \text{ER}/Cl_{free} \quad (13)$$

Therefore, changes in fu can affect steady state drug concentrations only through their influence on the total or free drug clearance. The relationships between fu and clearance will be different depending on whether the drug exhibits a low or high extraction ratio.

Low extraction ratio drugs

For drugs with low extraction ratios, Cl_{tot} is proportional to fu, and Cl_{free} is independent of fu, whatever the mechanisms of elimination are (Rowland & Tozer, 1995). This can be simply illustrated with drugs cleared only by the liver, for which the following equations can be used:

$$Cl_{tot} = \text{fu} \times Cl_{int} \quad (14)$$

$$Cl_{free} = Cl_{int} \quad (15)$$

where Cl_{int} is the intrinsic hepatic clearance, determined by the metabolic capacities of the liver.

Substituting eqns 14 and 15 into eqns 12 and 13, respectively, gives

$$C_{ss,tot} = \text{ER}/(\text{fu} \times Cl_{int}) \quad (16)$$

$$C_{ss,free} = \text{ER}/Cl_{int} \quad (17)$$

These relations indicate that for drugs with low extraction ratios, the steady state free concentration depends only on ER and Cl_{int} and modifications of fu will influence the steady state total concentration but not the steady state free concentration. This circumstance applies to most of the drugs used in veterinary medicine and will be the sole consideration in the following sections.

High extraction ratio drugs

In the case of drugs with high extraction ratios, the total clearance is independent of fu while the free clearance becomes dependent on fu. The effects of modifications of fu are described in Table 1. This situation does not apply to the majority of drugs encountered and is complicated by the influence of fu on systemic bioavailability when the drug is administered by oral route. Further details are given in a *Letter to the Editor* (*J. vet. Pharmacol. Therap.* (2002)**25:**239).

CONSEQUENCES FOR fu, C_{tot} AND C_{free} OF COMPETITIVE DRUG INTERACTION OR ALTERATION OF BINDING PROTEIN CONCENTRATION (LOW EXTRACTION RATIO DRUGS)

Competitive interactions

Several drugs can compete for the same plasma protein binding sites, leading potentially to drug displacements. Probably the best known example of such displacement is between warfarin and phenylbutazone (Aarons, 1981; McEInay, 1996). The competition for the same binding sites between a first drug of lower affinity termed the displaced (e.g. warfarin) and a second drug of higher affinity termed the displacer (e.g. phenylbutazone) is equivalent, from an operational point of view, to an increase in K_D for the displaced (i.e. a decrease of its affinity for the protein).

Table 1. The effects of altered protein binding on total and free drug concentrations in *in vitro* and *in vivo* situations

	Independent of fu	Dependent on fu	Variations	
In vitro	C_{tot}	C_{free}	fu↗	C_{free}↗
			fu↘	C_{free}↘
In vivo				
Low extraction ratio (most drugs)	C_{free}	C_{tot}	fu↗	C_{tot}↘
			fu↘	C_{tot}↗
In vivo				
High extraction ratio (few drugs)	C_{tot}	C_{free}	fu↗	C_{free}↗
			fu↘	C_{free}↘

When equilibrium is achieved, according to eqn 8, fu *increases* and according to eqns 16 and 17 (warfarin is a low extraction ratio drug), if fu increases then C_{tot} *decreases* but C_{free} remains unchanged (Fig. 1). C_{free} can only transiently increase until redistribution and elimination of the generally small amount of displaced drug occurs. Such redistribution and elimination phenomena make the *in vivo* situation radically different from an *in vitro* system, where these phenomena do not occur and C_{tot} is fixed experimentally (Fig. 1).

Concerning the drug–drug interaction between warfarin and phenylbutazone, the (actual) displacement of warfarin from its plasma binding sites by phenylbutazone is definitively not responsible for the increase in prothrombin time which is clinically observed. Moreover, eqn 17 explains why most drug–drug interactions having therapeutic significance are those which increase or decrease Cl_{int} – i.e. metabolic interactions – and it has been clearly established that the true origin of the pharmacological interaction between warfarin and phenylbutazone is the inhibition of metabolic processing of the S-warfarin enantiomer by phenylbutazone (Lewis *et al.*, 1974).

Modifications of plasma binding protein concentrations

When the plasma concentration of the binding protein increases, like for instance α1-acid glycoprotein during an inflammatory syndrome, then B_{max} increases (a particularly relevant situation for basic drugs, which bind to the α1-acid glycoprotein with high affinity). According to eqn 8, fu decreases and according to eqns 16 and 17, C_{tot} increases but again, C_{free} remains unchanged.

Conversely, when the plasma concentration of the binding protein decreases (e.g. albumin during nephropathy), fu increases, C_{tot} decreases and C_{free} remains unchanged.

Finally, for the large majority of drugs, interactions at the binding site or modifications of binding protein levels lead to an alteration of C_{tot}. This is relevant when interpreting a total

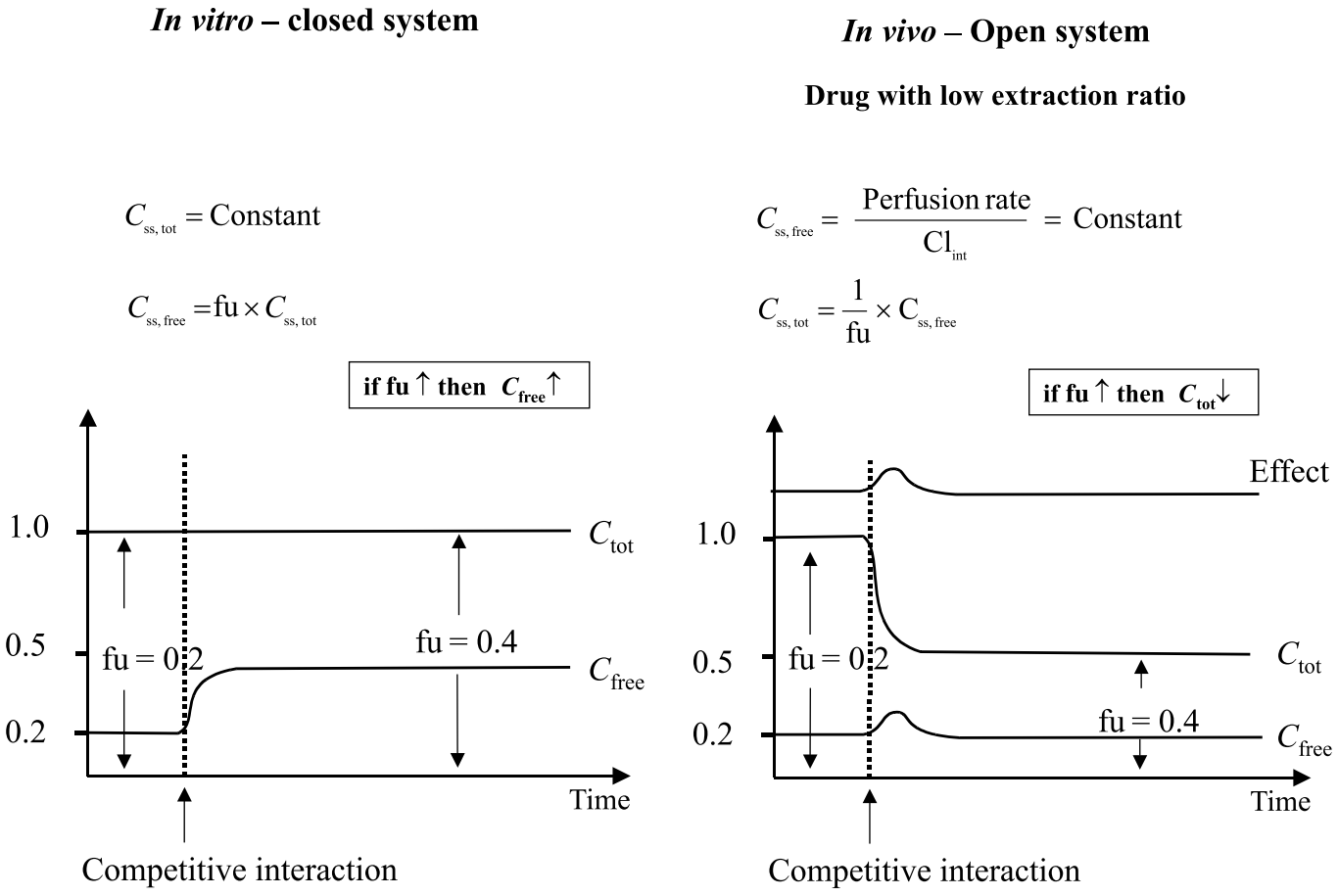

Fig. 1. Differential influence of plasma protein binding on the free concentration (C_{free}) and free fraction (fu). The relationships between the steady state free ($C_{ss,free}$) and total ($C_{ss,tot}$) drug concentrations are fundamentally different in an *in vitro* and an *in vivo* system. (Left panel) *In vitro*, the interaction between drug and binding protein occurs in a closed system. $C_{ss,tot}$ remains constant and an increase in fu because of displacement of the drug from its binding sites (arrow) is associated with an increase in $C_{ss,free}$. (Right panel) *In vivo*, steady state drug concentrations are controlled by the elimination process. For drugs with low extraction ratios, clearance of the free fraction is independent of fu and consequently the steady state free concentration ($C_{ss,free}$) remains unchanged when fu increases, whereas $C_{ss,tot}$ decreases. When competitive displacement occurs, there is only a transient increase in C_{free}, the small amount of drug displaced from the binding protein being rapidly (within a few minutes) redistributed and eliminated.

plasma concentration (therapeutic drug monitoring) but remains without consequence for the overall drug effect which depends only on C_{free}. Among exceptions to this general rule in veterinary pharmacology, are drugs against hematophagous parasites, such as lufenuron or salicylanilide drugs, for which the effects on, respectively, flea or fluke infection are directly controlled by the total concentration of drug ingested with blood.

CONCLUSION

As noted by others, drug binding displacement has been overestimated and overstated (Rolan, 1994; McEInay, 1996) and in the veterinary literature, there are many more articles with erroneous interpretations of drug binding than articles demonstrating an actual drug–drug interaction arising from an alteration of drug binding. Such erroneous interpretations may be avoided by recalling that *in vivo* the effects of modifications of fu on drug plasma concentrations are exerted only through modifications of total and/or free drug clearances.

Finally, there is now both a theoretical basis and experimental evidence that, for the large majority of drugs, plasma binding displacements do not affect the free drug concentration in the steady state and hence are without consequence for the overall drug effect. The main area in which drug binding should be taken into account is that of therapeutic drug monitoring, as a displacement can alter C_{tot} without affecting C_{free}, risking a recommendation or decision by clinicians to increase, without justifications, dosage regimen.

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