PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

On the Possibility of Self-Induction of Drug Protein Binding

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ABSTRACT: The equilibrium unbound drug fraction (f_u) is an important pharmacokinetic parameter, which influences drug elimination and distribution in the body. Commonly the drug plasma concentration is substantially less then that of drug binding proteins, so that f_u can be assumed constant independent of drug concentration. A general consideration of protein binding based on the mass-action law provides that the unbound drug fraction increases with the increase of drug concentration, which is also a usual experimental observation. For several drugs, though, a seemingly unusual sharp decrease of the unbound drug fraction with the increase of total drug concentration (R_o) in the interval $0 < R_o \lesssim 5\,\mu M$ was experimentally observed. A possible explanation of this apparently strange phenomenon is presented. The explanation is based on the consideration of a two-step mechanism of drug protein binding. The first step occurs as a drug binding to the site with relatively low affinity. Consequently this binding leads to the activation of a high affinity site, which otherwise is not available for binding. The suggested binding scheme yields the curves for f_u dependence on the total drug concentration that are in good agreement with experimental measurements. The interpretation of pharmacokinetic data for the drugs with such unusual concentration dependence of f_u appears to be a formidable problem. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:4400–4405, 2010

Keywords: anomalous protein binding; unbound drug fraction; concentration dependence of the unbound drug fraction; two-step protein binding; allosteric activation; self-induction; pharmacokinetics

INTRODUCTION

Binding of drugs to plasma proteins is the feature that influences drug pharmacokinetcs and its pharmacological activity.[1,2] Most often the concentration of drug in plasma is much less than that of a binding protein, so that the dependence of the unbound drug fraction in plasma, f_u, on drug concentration is negligible, and f_u is assumed as constant value f_u^o. In general, drug-protein binding is treated as a set of simple binding reactions to each site of each protein involved in binding, which are commonly albumin and α_1-acid glycoprotein (AGP). For such a consideration, the increase of total drug concentration, R_o, results in a gradual increase of f_u from $f_u^o = f_u(R_o \to 0)$ to $f_u \to 1$ when $R_o \to \infty$.[3] A seemingly unusual phenomenon of a strong decrease of f_u with the increase of total drug concentration was experimentally observed for several drugs at low concentrations. The unbound fraction was dropping from the values between 50 to 100% at drug concentrations around or less than 1 μM to the "regular" almost constant values around 2–20% when measured at higher drug concentrations in the interval $5\,\mu M \lesssim R_o \lesssim 40\,\mu M$. This apparently strange concentration dependence of f_u was observed both in vitro and in vivo and reported in literature for profolol,[4–6] hexanoic, decanoic and dodecanoic acids,[7] indomethacin,[7] and lidocaine.[7] Lidocaine binds mostly to AGP, while other mentioned drugs bind mostly to albumin. It was suggested that the presence of hydration layer (well-structured water) around protein molecule is probably the main reason leading to the observed anomalous concentration changes of the free drug fraction.[6,7] This explanation appears reasonable and attractive. Though the analysis of the corresponding binding

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scheme, that would validate the relevance of the suggested hypothesis, was not done. As shown below, a detailed consideration of drug protein binding, which includes the replacement of water from the binding site, does not provide the observed decrease of f_u at low drug concentrations. The binding of a drug to the hydrated site (i.e. occupied by n water molecules) is described by the reaction

$$P\text{-}nH_2O + R \rightleftarrows C + nH_2O \quad (1)$$

where P denotes the protein molecule, R - the drug molecule, and C = P-R signifies the drug protein complex. The reaction above can be treated as a sum of two reactions, i.e. the hydration reaction and the drug binding reaction to the free (dehydrated) protein

$$P\text{-}nH_2O \rightleftarrows P + nH_2O \quad (2)$$

$$P + R \rightleftarrows C \quad (3)$$

The equilibrium of reactions, Eqs. (2) and (3), is determined by the mass action law

$$K_h = \frac{[P][H_2O]^n}{[P\text{-}nH_2O]} \quad (4)$$

$$K_d = \frac{[P][R]}{[C]} \quad (5)$$

where K_h is the equilibrium constant of the hydration reaction (Eq. 2) and K_d is the equilibrium dissociation constant (affinity) of drug protein complex (Eq. 3). Applying the mass balance equations $P_o = [P] + [C] + [P\text{-}nH_2O]$ and $R_o = [R] + [C]$, where P_o is the total protein concentration, yields the following equation for the unbound drug fraction $f_u = [R]/R_o$ (Appendix)

$$f_u^2 - f_u\left[1 - \frac{P_o}{R_o} - \frac{K_d}{R_o}(1 + F_h)\right] - \frac{K_d}{R_o}(1 + F_h) = 0 \quad (6)$$

where $F_h = [H_2O]^n/K_h$. The absence of hydration corresponds to $K_h \to \infty$ (i.e. $[P\text{-}nH_2O] = 0$ as follows from Eq. 4). Then Eq. (6) for f_u becomes exactly the same as for a simple binding reaction that is described by Eqs. (3) and (5) with the equilibrium dissociation constant K_d. The account of possible hydration just results in the replacement of K_d by "effective" dissociation constant $K_d(1 + F_h)$, which is reflected in Eq. (6) for f_u, and eventually yields larger value of $f_u^o = 1/[1 + P_o/(K_d(1 + F_h))]$ compared to that $f_u^o = 1/[1 + P_o/K_d]$ in the absence of hydration.[3] This is shown in Figure 1 for f_u calculated by Eq. (6) (Eq. A6) for the binding occurring by dehydration scheme (Eqs. 1–3, $F_h > 0$ in Eq. 6), and by direct simple binding (Eq. 3, $F_h = 0$ in Eq. 6). Consideration of possible hydration hindrance of binding suggests that it would lead to the increase of f_u compared to that provided by direct binding, and that f_u would be gradually increasing with the increase of total drug concentration. Similarly, the consideration of drug binding to the protein which has N binding sites of the same affinity K_d, would just lead to the replacement of P_o by NP_o in Eq. (6), so that f_u would still be the increasing function of the total drug concentration as shown in Figure 1. Thus the hindering effect of hydration layer does not seem to explain the experimental observations of the anomalous concentration dependence of f_u.

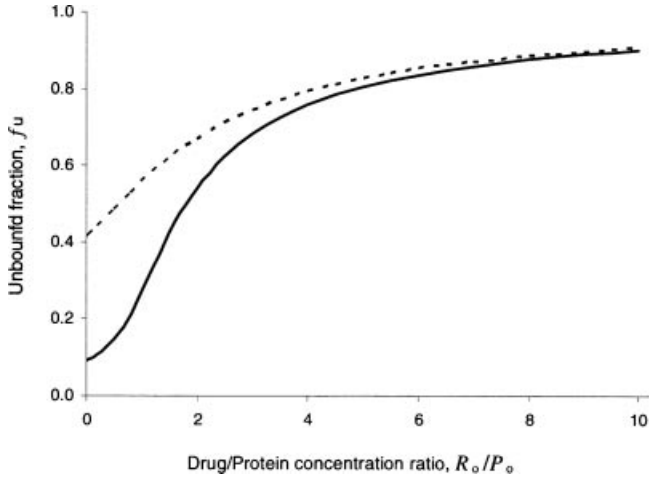

Figure 1. The dependence of the unbound drug fraction on total drug concentration in the presence and absence of hydration. Calculation is performed by Eq. (6) (Eq. A6) with $P_o/K_d = 10$, $F_h = 6$ for the presence of hydration (— —), and $F_h = 0$ for the direct simple drug protein binding (—).

CONSIDERATION OF SELF-INDUCTION MECHNISM OF DRUG PROTEIN BINDING

To explain the unusual concentration dependence of $f_u(R_o)$ we suggest the following conceivable two-step mechanism of drug protein binding. The first step occurs as drug binding to the site with relatively low affinity, characterized by the equilibrium dissociation constant K_{d1}. Consequently this binding leads to the activation of a high affinity site with equilibrium dissociation constant K_{d2}, which is otherwise not available for binding. Such kind of binding mechanism corresponds to allosteric interaction, which results in protein modification caused by a drug. In other words the drug molecule serves as allosteric activator upon binding of which to low affinity site, the binding to high activity site would take place. It is plausible that binding to low affinity site (or several sites) leads to some conformational rearrangement of the protein molecule consequently resulting in the loss or weakening of the hypothetical well-structured water barrier around high affinity site. The suggested