Special Populations: Protein Binding Aspects

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Contents

Abstract

The aim of this chapter is to provide a basic understanding of the effect of protein binding and its alterations on the pharmacokinetics of drugs and their pharmacological and clinical effects. This has been matter of controversies in the last decades: this chapter calls for scientific comprehension of the underlying phenomena and recommends a reasoned experimental program aiming to the characterization of the protein binding of new compounds and the effects of its changes in different conditions. Considerations and data are also reported concerning the potential changes of drugs' protein binding in some pathophysiological conditions.

Introduction

Background

In addition to proteins specifically acting as transport proteins (e.g., sex hormone binding protein, cortisol-binding globulin), many proteins can bind ligands, such as endogenous compounds or xenobiotics. Among the most important proteins able to bind drugs, there are albumin, α_1-acid glycoprotein (AAG), and lipoproteins (Hervé et al. 1994). Human albumin is responsible for the binding of drugs with different physical-chemical characteristics, but it has affinity especially for acidic drugs. It is characterized

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by a molecular weight of 67 kDa and accounts for more than one half of the plasma proteins in humans (normal values for albumin concentration are 3.5–5.5 g/dL, i.e., approximately 500–750 μM); therefore, it provides the higher binding capacity for ligands (Mehvar 2005; Zhang 2012). AAG, with molecular weight 42 kDa, binds preferentially basic or neutral drugs and it is present in human plasma at much lower concentrations (1–3% of total plasma proteins; normal values are 0.04–0.1 g/dL, i.e., approximately 9–23 μM) (Mehvar 2005); for this reason, the binding of drugs to this protein can be more easily saturated. Lipoproteins are proteins able to bind basic or neutral drugs characterized by high lipophilicity. They are classified based on their density (high, low, and very low) and characterized by variable molecular weight (≥200,000). Their plasma concentrations are variable, lower than 0.5 g/dL (Mehvar 2005).

All drugs, when they are in the systemic circulation of animals or human subjects, are characterized by some degree of interaction with plasma proteins. The interaction can be negligible (for instance, the case of acethaminophene, atenolol, carboplatin, ethosuximide, metformin, and ribavirin), therefore providing the total drug concentrations essentially in the unbound form (Rowland et al. 2011a; Zhang et al. 2012). Other drugs are avidly bound to plasma proteins, which, in turn, results in very small unbound concentrations of the compound in the systemic circulation (<1% of the total drug concentrations; for instance, amiodarone, diclofenac, isotretinoin, ketoprofen, nabumetone, naproxen, and teniposide) (Rowland et al. 2011a; Zhang et al. 2012).

Kinetics and Dynamics of the Drug-Protein Interactions

The interaction between drugs and protein is occurring typically in milliseconds and a variety of parameters or metrics can be used to describe the extent of binding (Rowland et al. 2011b). The fraction unbound (fu, expressed as such or as %), defined as the ratio between the free and the total (i.e., the sum of free drug and protein-drug complex concentrations) drug concentration:

$$fu = \frac{[free\ drug]}{[free\ drug] + [protein - drug]}$$

should be considered the preferred metric to be used to describe protein binding. In this ratio, [*free drug*] is the concentration of unbound drug and [*protein − drug*] is the concentration of the protein-drug complex; the complement of fu to unit (fraction bound or protein binding, also expressed as such or as %) is also used.

It should be considered that the interaction between proteins and drugs can be described in the light of the mass balance law: the relationships between free protein and ligand concentrations and the complex protein-ligand concentration can be described as a chemical equilibrium and therefore ruled by a dissociation (or association) constant.

$$protein - drug \underset{\leftarrow}{\overset{\rightarrow}{}} free\ protein + free\ drug$$

$$k_D = \frac{k_{\rightarrow}}{k_{\leftarrow}} = \frac{[free\ protein]\cdot[free\ drug]}{[protein - drug]},$$

where k_D is the dissociation equilibrium constant, which can also be seen as the ratio of the kinetic micro-rate constants, [*free protein*] is the concentration of free protein, and the other terms were defined above. This equation is appropriate in case the protein-drug complex has a 1:1 stoichiometry; other forms of the mass balance equation can be devised depending on the stoichiometry of the protein-drug complex. Based on the above equation, it can be easily understood that fu is not, in principle, invariant with total drug concentrations: it can be considered invariant when conditions far from saturation are realized (i.e., when total drug concentrations are much lower than total protein concentrations). Saturation, which is rarely approached for drugs binding to albumin, can sometimes be achieved when drugs are avidly bound to AAG.

Impact of Altered Unbound Exposure

It is important to notice that protein binding deals with the ability of crossing membranes; the higher the unbound concentrations the higher can be the interaction with the target responsible for drug effects (both clinical efficacy and clinical safety) and the interaction with entities responsible for drug disposition (for instance, the drug-metabolizing enzymes in hepatocytes). In general, however, when unbound drug concentrations account for $\geq$10% of total drug concentrations, the effect of protein binding on the membrane diffusion is relatively unimportant, as there is always a sufficient amount of free drug that can cross membranes and interact with targets (Rolan 1994). While this should be applied to actual measurements of unbound and total drug concentrations, this is often applied to unbound data assessed in separate experiments. Thus, unbound concentrations can be considered equal to the product fu·total drug plasma concentration or when the systemic exposure is expressed in terms of area under the plasma concentration-time curves (AUC), $AUC_{unbound} = fu \cdot AUC_{total}$.

Changes in plasma protein binding are considered important in most cases by many scientists (Musteata 2012; Ascenzi et al. 2014); other groups, not without reason, are instead stating that protein binding changes are clinically meaningful in a minority of specific cases (Rolan 1994; Benet 2002; DeVane 2002). The latter opinion is largely based on physiological considerations related to the disposition of compounds. In this regard, we can assume, in first approximation, that the unbound AUC is responsible for the drug effects.

$$AUC_u = f_u \cdot AUC = f_u \cdot \frac{Dose}{\frac{CL}{F}},$$

where CL is the elimination clearance of the drug and F is the absolute bioavailability of the drug (CL/F is thus the apparent clearance of the oral drug); the other terms are defined previously in the text. Assuming also that drugs are eliminated exclusively via hepatic metabolism, hepatic CL (CL_H~CL) can be accurately described by the well-stirred model (Wilkinson and Shand 1975; Pang and Rowland 1977)

$$CL_H = \frac{Q_H \cdot f_u \cdot CL_{int}}{Q_H + f_u \cdot CL_{int}}$$

where Q_H is the hepatic blood flow, fu is the fraction unbound (here expressed in blood), and CL_{int} is the hepatic intrinsic clearance, which represents the intrinsic ability of the liver to remove the drug from the systemic exposure in the absence of blood flow limitations and binding (Wilkinson and Shand 1975). It can also be assumed that the drug is completely absorbed from the gastrointestinal tract and that there is no metabolism in the gut (i.e., the absolute bioavailability is equal to the hepatic bioavailability, F~F_H). Hepatic bioavailability (i.e., the fraction of dose that escapes the hepatic first pass) can be written as:

$$F_H = 1 - \frac{CL_H}{Q_H} = \frac{Q_H}{Q_H + fu \cdot CL_{int}},$$

where all the terms were already defined above.

For drugs given orally, rearranging the above relationships, it is easy to demonstrate that the unbound exposure is independent of fu:

$$AUCu = fu \cdot \frac{Dose}{\frac{Q_H \cdot f_u \cdot CL_{int}}{Q_H + f_u \cdot CL_{int}} \Big/ \frac{Q_H}{Q_H + fu \cdot CL_{int}}} = \frac{Dose}{CL_{int}}$$

For drugs given intravenously, F = 1 by definition, so that the following relationship can be written:

$$AUCu = fu \cdot \frac{Dose}{\frac{Q_H \cdot f_u \cdot CL_{int}}{Q_H + f_u \cdot CL_{int}}}$$

In this case, the outcome is different for drugs characterized by high hepatic extraction (i.e., drugs for which fu.CLH> > QH) versus low

hepatic extraction (i.e., drugs for which fu. CLH $<<$Q). In the first case, Q_H in the $Q_H + f_u \cdot CL_{int}$ term can be considered negligible, so that the terms fu.CL_{int} can be simplified and the unbound exposure is effectively dependent on fu:

$$AUC_u = fu \cdot \frac{Dose}{Q_H}$$

Vice versa, in the second case, the term fu. CL_{int} can be considered negligible, so that Q_H and fu can be simplified and the unbound exposure is effectively not dependent on fu:

$$AUC_u = \frac{Dose}{CL_{int}}$$

Therefore, the binding of a drug is relevant in terms of its influence on the clinical effects only in case of intravenous administration of drugs with high extraction ratio. Other cases in which meaningful clinical effects are expected for low hepatic extraction drugs are in case of transient increase in free drug concentrations (Benet 2002; Rolan 1994). Benet (2002) indicates that the above considerations can be extended to drugs characterized by nonhepatic clearance. Also in this case, the effect of protein binding is clinically relevant in case of drugs with high extraction ratio given intravenously. The same consideration should hold true for drugs with high nonhepatic extraction ratio given orally, but the author claimed that no drugs from a consulted list of 452 drugs, eventually met these criteria.

With respect to the volume of distribution (V, assuming expressed in terms of blood concentrations), it can be written (Rowland et al. 2011c):

$$V = V_B + \frac{f_u}{f_T} \cdot V_{TW},$$

where V_B is the volume of blood, f_u is the fraction unbound in blood, f_T is the fraction unbound in tissues, and V_{TW} is the aqueous volume outside the blood. For drugs with sufficiently large V, V_B can be neglected and V becomes proportional to f_u. Based on the fact that the half-life ($t_{1/2}$) can be expressed as:

$$t_{1/2} = \frac{\ln(2) \cdot V}{CL}$$

the considerations done above for CL can be further extended.

To some extent, however, these are theoretical pharmacokinetic considerations, sometimes valid for asymptotic situations, supported more by the lack of observed clinical effects. A general recommendation is that the pharmacokinetics of new candidate drugs, especially those with high plasma protein binding, should always be performed measuring both total and unbound species in the clinical pharmacology trials, to increase the clinical PK understanding of the new molecule.

Change of Proteins Concentrations in Special Populations

In the following section, information on the protein concentration and protein binding in different special populations will be given. The relevancy of such changes should though be assessed in the light of the above-mentioned considerations.

Pediatric and Elderly subjects

Neonates and infants tend to have reduced concentration of plasma proteins compared to adult subjects (Lu and Rosenbaum 2014). Serum albumin concentrations at birth are approximately 75%–80% of those measured in adults; AAG concentrations, instead, account for approximately one-half of the concentrations in adults (McNamara and Alcorn 2002). Protein concentrations eventually achieve adult levels at 1–3 years of age. In addition to changes in plasma protein concentrations, fetal albumin is characterized by a lower binding affinity to drugs compared to human adults, indicating that also the ability of albumin to bind drugs may be lower in pediatric subjects (Lu and Rosenbaum 2014). Because of these finding, the fraction unbound of drugs are typically higher in pediatric subjects than in adults and protein binding is, therefore, reduced. A paper

(McNamara and Alcorn 2002) reports fu data for a list of 39 drugs bound to either albumin or AAG. The average fraction unbound in adults of the drugs reported in this dataset is lower (approximately 67%) compared to the average of those observed in neonates at term. The same paper reports an interesting computational model, based on simple mass balance equation concepts, that was able to predict with reasonable accuracy fu in plasma of pediatric subjects based on the fu obtained in adults and the relative concentrations of proteins (P) in pediatric and adult subjects:

$$fu_{pediatric} = \frac{1}{1 + \frac{P_{pediatric}}{P_{adult}} \cdot \frac{(1 - fu_{adult})}{fu_{adult}}}$$

This approach could be easily extended also to the other special populations.

In the aging subjects, albumin concentrations are slowly decreasing with age. Albumin concentrations in an 80-year-old subjects are approximately 20% lower than those observed in 20-year subjects (Wallace and Verbeeck 1987). Applying the above relationship indicated by (McNamara and Alcorn 2002) to a drug with fraction unbound of 0.10 in adults, the fraction unbound in an 80-year subjects would be predicted to increase approximately to 0.12, i.e., likely within the inter-subject variability of fu in adult subjects. Although the dependency of AAG is affected by larger variability, it seems that the plasma concentrations of this protein are instead slightly increasing with age. In an extensive review, the protein binding values for elderly were tabulated for more than 50 drugs in elderly subjects (Grandison and Boudinot 2000); selected data from this report are shown in Table 1.

It is interesting to notice that, for diazepam, oral clearance is relatively independent of age; however, this is the result of the counterbalancing of the increased fu with a decreased CLu, likely due to the decreased metabolic clearance in elderly subjects, as reported in Rowland et al. (2011d).

Only approximately 30% of the (Grandison and Boudinot 2000) dataset was composed by drugs with fu <0.1. From the table reported in the original paper, it can be appreciated that more than 50%, approximately 28%, and less than 10% of the compounds showed no relevant changes, an increase and a decrease in the fu values in elderly compared to adults, respectively.

Pregnant Women

Numerous physiological changes occur during pregnancy. Plasma volume may expand up to 50%. The plasma expansion is quicker than the albumin production which leads to physiological hypoalbuminemia due to dilution and thus decreased binding. Plasma albumin concentrations were reported to decrease on average from 44 g/L in the third month of gestation to 32 g/L in the ninth month of gestation (Notarianni 1990). This effect may be further enhanced by the decreased binding capacity due to the increased circulating concentrations of steroid and hormones. As indicated in the introduction, however, the increased free drug concentration is compensated by an increased clearance in many cases (Loebstein et al. 1997).

Some conflicting results have been reported in terms of the effect of pregnancy on AAG concentrations: in some cases, no changes have been reported (Loebstein et al. 1997; Chu et al. 1981), while in other cases, a decrease, though smaller than that reported for albumin, was observed (Notarianni 1990); also in this case, concentrations declined with the month of gestation (from 0.72 g/L in the third month of gestation to 0.50 g/L in the last month of gestation). In case of pregnancy complications (e.g., preeclampsia), AAG was found to significantly increase (Chu et al. 1981).

Hepatic Impairment

Liver disease may decrease protein binding of drugs (or, in turn, increase fraction unbound) via decreased protein concentrations due to reduced synthesis of albumin and AAG and accumulation of endogenous inhibitors which may compete for the binding (Verbeeck 2008). Fraction unbound of individual compounds may show significant

Table 1 Fraction unbound (fu) data in adult and elderly subjects for selected drugs (extracted from (Grandison and Boudinot 2000))

Compound	Population	fu	
		Adult	Elderly
Acetazolamide	Healthy	0.041	0.069
Alfentanil	Gastrointestinal surgery	0.093	0.093
Amitriptyline	Healthy	0.052	0.044
Benazeprilat	Healthy	0.083	0.106
Canrenone	Healthy	0.050	0.060
Diazepam	Healthy males Healthy females	0.0125 0.0134	0.0172 0.0166
Diflunisal	Healthy	0.0012	0.0019
Digitoxin	Healthy	0.041	0.045
Flurazepam	Healthy	0.031	0.037
Haloperidol	Healthy	0.095	0.085
Ibuprofen (S) Ibuprofen (R)	Healthy	0.0059 0.0039	0.0078 0.0040
Naproxen	Healthy	0.00084–0.0023	0.0017–0.0051
Nitrazepam	Healthy males Healthy females	0.178 0.179	0.189 0.190
Triazolam	Healthy males Healthy females	0.213 0.229	0.247 0.228
Verapamil	Healthy	0.09	0.10

Table 2 Protein binding data in studies in subjects with hepatic impairment

Compound	Fraction	%				Reference
		Normal healthy subjects	Mild hepatic impairment	Moderate hepatic impairment	Severe hepatic impairment	
Dabigatran	Bound	28.8 ± 1.55	34.5 ± 3.65[a]			(Stangier et al. 2008)
Everolimus	Bound	73.8 ± 3.6	73.5 ± 2.4			(Kovarik et al. 2001)
Febuxostat	Unbound	0.7	0.7	0.6		(Khosravan et al. 2006)
Lopinavir	Unbound	0.69 ± 0.06	0.89 ± 0.21	0.94 ± 0.10	0.91 ± 0.16	(Peng et al. 2006)
Sildenafil	Unbound	3.46 ± 0.61	3.70 ± 1.34			(Muirhead et al. 2002)
Tiagabine	Unbound	3.59 ± 0.74	3.11 ± 0.32	5.13 ± 0.89		(Lau et al. 1997)
Ziprasidone	Bound	99.92 ± 0.03	99.84 ± 0.11	99.91 ± 0.04		(Everson et al. 2000)

[a]Different from normal healthy subjects, but similar to the value of 35% obtained in other phase 1 clinical trials in healthy volunteers and reported in the label

When available, data are reported as mean ± SD

increases, whilst no important changes are reported for others. Data for selected drugs are summarized in Table 2.

Renal Impairment

Protein binding to albumin is often decreased in patients suffering from an impaired renal function. As in the case of subjects with liver impairment, hypoalbuminemia, accumulation of endogenous substances competing for the binding sites or conformational alteration of the albumin molecule may be the leading cause of the changes of protein binding. Binding to AAG may instead increase due to the higher α1-acid glycoprotein concentration observed in certain categories of patients (renal transplant, patients undergoing hemodialysis). Unbound fraction data for selected drugs are summarized in Table 3.

Table 3 Fraction unbound (fu) data in subjects with hepatic impairment

Compound	Fraction	%				Reference
		Normal subjects	Mild hepatic impairment	Moderate hepatic impairment	Severe hepatic impairment	
Diazepam	Unbound	4.2 ± 0.5 4.6 ± 0.8	4.4 ± 0.7		5.4 ± 1.1[a]	(Viani et al. 1992)
Digitoxin	Unbound	4.6 ± 0.7 5.2 ± 1.0	4.7 ± 0.8		5.9 ± 0.7[a]	(Viani et al. 1992)
Salycilic acid	Unbound	26.0 ± 5.4 34.2 ± 4.6	29.3 ± 5.1		36.3 ± 5.1[a]	(Viani et al. 1992)
Sildenafil	Unbound	2.7 ± 0.8	2.4 ± 0.7	2.0 ± 0.5	2.2 ± 0.3	(Muirhead et al. 2002)
Valproic acid	Unbound	8.4 ± 2.5	20.3 ± 4.7			(Gugler and Mueller 1978)

[a]Subjects undergoing emodialysis
mean ± SD

Oncology Patients

Oncology patients typically have decreased albumin and increased AAG plasma concentrations. Genentech scientists initiated the effort of building a database of the demographic and physiological characteristics of oncology patient to be used in the development of physiology-based pharmacokinetic modeling (Cheeti et al. 2013). In this effort, they also characterized the distribution of plasma proteins in a population of oncology patients (N = 2597) from phase 1, 2, and 3 trials. The distribution of albumin plasma concentrations in their database had a peak at 38 and 41 g/L in male and female subjects, respectively (with values ranging from 3 to 50 g/L), while the typical value observed in healthy subjects is approximately 45 g/L. Likely due to potential inflammation processes in this patient population, the AAG concentrations in the oncology patients of their database were higher than in healthy subjects. The AAG plasma concentration distribution peaked at 1.3 g/L (range 0.3–4 g/L) compared to a typical value of 0.8 g/L reported in healthy subjects.

Conclusions

The effect of protein binding and of its alterations on the pharmacokinetics of drugs has been the matter of numerous controversies in the last decades. Based on the understanding of the pharmacokinetics of drugs, it seems likely that this is relevant only in a relatively restricted number of cases, for instance, for drugs with fraction unbound <0.1 and characterized by high clearance. However, a scientific approach should always be adopted, which requires: (i) to assess the pharmacokinetics of total and unbound concentrations of a new chemical entity and, possibly, avoid reference to fu measurements obtained in separate (ex-vivo) experiments, (ii) consider unbound concentrations at the receptor site to assess the potential effect of a change of unbound fraction on the drug effects, and (iii) always exert clinical judgement, especially for the effects that can be observed in specific group of patients requiring special attentions, such as subjects with severe organ impairment, infants, frail elderly, critically ill subjects, etc. (Roberts et al. 2013; Schmidt et al. 2010).

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