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The role of the protein-binding on the mode of drug action as well the interactions with other drugs

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SUMMARY

Drug transport and disposition are influenced by a non-specific and reversible drug binding to plasma and tissues proteins. Albumin and a1 acid glycoprotein are the most important transport proteins of the blood. Albumin possesses specific sites for acidic and basic drug binding and can interact with them in the plasma since a third site is trapped only by digoxin. Diseases and stress conditions induce conformational changes either in plasma or in tissue proteins by the synthesis of endogenous substances which can strong interfere with the amount of the free pharmacological effective drug ratio. This may affect the binding of drugs in target molecules inducing significant pharmacokinetic alterations. Stress conditions are associated with FFA increase in serum playing an antagonistic role with other acidic molecules (e.g. ampicillin) to the same binding site. The bounded drug is displaced and freer ratio is available to interact with various organ receptors leading to pharmacological effect enhancement and therefore to side effects manifestation such as seizures. Furthermore conjunctive tissues diseases, ageing, prolonged bleeding, starvation or diseases affecting protein profile, characterized by reduced total plasma proteins, followed by albumin decrease and lessen binding sites lead to more free drug availability enhancing its pharmacological effect. Increased a_1-acid glycoprotein the acute phase protein as by heart infraction or liver morbidities (e.g CCl_4 intoxication) mainly occupied from basic substances, in the case of cationic drug treatment resulted to the enhancement of them and consequently to pronounced effectiveness. In addition, renal failure reduced free fractions of many acidic drugs. It may be concluded that by narrowed therapeutic index of a medicine, and when drug/drug or drug/disease interactions are anticipated, drug monitoring seems to be necessary for its dosage adjustment.

INTRODUCTION

Theoretical and experimental data of drug binding to serum and tissues protein lead to clinical relevance of the role of the bound/unbound drug fraction in the development of interactions process.

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FACTORS RELATED TO ALBUMIN

Drug transport and disposition in the organism are dynamic processes, since drugs are non-specifically and reversibly bound to plasma and tissues proteins. Although various molecules bind to most proteins in blood (blood cells and serum proteins), human serum albumin (HSA), having molecular weight of 66 KD, is the most important transport protein with a very high capacity for binding endogenous and exogenous compounds in plasma (1). HAS is the most abundant plasma protein, possessing multiple hydrophobic

binding sites (a total of eight for fatty acids and endogenous ligands and binds neutral and negatively charged molecules). HAS consists of three homologous α-helical domains (I-III). Furthermore, the protein possesses two (I and II) major and selective drug binding sites, known as Sudlow's sites in sub domain IIA and IIIA, while a third site (III) is occupied only from digoxin (2,3). Albumin can interact with acidic or basic drugs (warfarin, ibuprofen, diazepam) and endogenous substances in the plasma by van der Waals dispersion and hydrophobic forces, hydrogen bonding, ionic interactions and other attractive forces as well (4). Protein binding values (% fraction bound) are normally given as the percentage of the total plasma concentration [Dt], of a drug that is bound to all plasma proteins [DP], while an amount remains free [D_f] (1).

Total Plasma Concentration [Dt] = [D_f] + [DP]

The degree of drug - plasma protein binding (drug-protein binding complex) gives significant information on the pharmacokinetic and the pharmacodynamic properties of a drug or better it can determine and specify the drug distribution. Considering the high concentration of albumin and the variable range of effective therapeutic levels of drugs, from nM to mM, the free drug fraction available for therapeutic action is drastically reduced for drugs with high protein binding e.g. drugs with high affinity for the protein (>95% bound) require correspondingly higher doses to achieve the effective concentration *in vivo,* are slowly distributed to sites of action and may not be timely eliminated. However, the affinity of drugs for plasma proteins is less than that for their receptors or enzyme targets. The drug-protein complex represents a silent deposit of free drug fraction; since the bound drug is unable to exert any effect such, for instance to penetrate the wall of the blood vessels, to interact with receptors, to be metabolized or to be excreted; thus prolonging its duration of action. Furthermore, drugs with high affinity for plasma proteins are able to compete with other agents for the same protein binding site. Additionally, since the unbound substances are the pharmacologically active moieties, albumin may serve as a circulating deposit (5,6).

Thus, the protein binding can affect the apparent volume of distribution as well as the hepatic and renal clearance and therefore, may change the ratio of free *per* total drug in plasma without modification of the pharmacodynamic result. For this reason protein binding represents an important parameter for the total drug assessment in the clinical drug monitoring procedures. Furthermore, binding is variable, since once the free fraction is reduced, the drug-protein complex offers free drug to the circulation thus obtaining a constant equilibrium between free and bound drug at any time.

(free drug) + (albumin) $\underset{k_2}{\overset{k_1}{\rightleftharpoons}}$ (drug-albumin complex)

where k_1 and k_2 are the association and dissociation rate constants, respectively.

The biological properties of a protein depend on its conformation while the latter can be influenced by physical or chemical factors, e.g. ligand binding. The extent of drug binding to serum proteins is strongly influenced by the pH and the temperature (7,8).

Complexities due to interactions between drugs and endogenous ligants often occur *in vivo* for HAS (9-12).

Concerning binding site II, it is more sensitive for basic molecules but upon saturation of site I, it can bind acidic compounds as well. Drugs or endogenous substances can compete for the same binding site to albumin. The binding extent of different compounds to protein is dependent on their affinity. Co-administered drugs may exert a competition for the binding to the same protein site, then a displacement of the drug with the limited binding affinity occurs and its non-bound, free fraction is enhanced. Similarly, competition for a certain binding site may occur between drugs and endogenous substances (12).

Usually site I of albumin is recognized as warfarin site because, when warfarin is co-administered with other compounds e.g. NSAIDs or acidic antibiotics (β-lactams) or under the presence high concentration of endogenous substances (FFA) its free fraction is enhanced by its displacement from these substances (13-15). The clinical consequence of this event is the enhancement of warfarin active concentration leading to increased pharmacological effects, such as hemorrhagic diathesis. This pronounced effect due to the displacement process is attributed to the narrow therapeutic index of warfarin.

Site II is called the diazepam site because in situations of diazepam co-administration with other drugs (in principle basic substances), its free fraction is increased via displacement from its binding sites and prolongation of sedative effect. However, this enhanced diazepam levels produce no severe side effects because of its wide therapeutic index. Furthermore, in recent reports it is mentioned that albumin possesses a third binding site III, yet with limited importance.

On the other hand a1 acidic glycoprotein (AGP), an acute phase protein, interacts mainly with basic entities whereas lipoproteins bind both basic and neutral

drugs. However there is evidence for binding of certain acidic drugs to AGP as well (16). Increased synthesis of AGP occurs in response to stress, infections including the acquired immune deficiency syndrome (AIDS), burn injury, myocardial infraction, rheumatoid arthritis, chronic pain, uraemia and malignancy. Albumin, despite its large size is not exclusively retained in the plasma but it can be distributed extravascularly (17,18).

DISEASES INFLUENCE DRUG- PROTEIN BINDING

In acute diseases, albumin is reduced and an increase of γ-globulin is observed as a host reaction to injury or infection. A great number of diseases lead to decreases in albumin while conditions like schizophrenia or optic neuritis are coupled to increased albumin concentration (19,20).

It has already been demonstrated, that stress as a synchronized stimulation of sympathetic and endocrine systems, leads to excessive increase of free fatty acids. Surgical operations, trauma, experimental procedures or drug administration are associated with decreased drug binding capacity in humans and in laboratory animals, due to hypoalbunaemia and serum FFA increase. As mentioned above, FFA enhancement represents a stressful condition leading to an impairment of binding affinity to the site 1 of albumin (21).

An extremely large number of diseases and physiological states (pregnancy) may lead to alteration in FFA and (mostly lowering) of the plasma albumin concentration. Serum fatty acids levels of 0.1 and 2 mol per mol of HSA can compete and cooperate with drugs binding to the protein. In certain diseases, these effects are extreme as the fatty acid/HSA mole ratio may rise to six (22). Interacting with fatty acids, HSA molecules undergo dramatic conformational changes as shown in crystallographic studies (15,23,24).

Other pathological conditions with high levels of bilirubin, hemin, or renal toxins which bind to the protein, cause significant drug binding defects (20,25,26). Furthermore excess steroid levels (i.e. during pregnancy, steroid treatment etc.) in addition to long fatty acids chain can allosterically modulate albumin binding properties (27-30).

Forced swimming in cold water (4 °C) of Wistar rats is referred to as a model of mental distress in association with corporal exhaustion resulting to disturbances on serum lipid profile (31-33). In a model of chronic stress, ampicillin levels were measured in relation to stress duration and directly related to serum FFA levels. It is obvious that although ampicillin is a relatively safe drug, in high concentrations it may manifest side effects for instance from the CNS (seizures) (34,35). In a model of surgical trauma in rats treated with quinolones we have reported increased quinolone concentration in tissues. Moreover in the same model, when ibuprofen was co-administered with quinolones the latter were increased in both serum and tissues (36,37). Comparable results were observed in a hyperlipidaemic experimental model, where Wistar rats were treated with cephalosporins simultaneously with ibuprofen. Cephalosporins were increased in fat fed animals probably via displacement of albumin binding site by the circulated lipoproteins and FFA as well as by the presence of ibuprofen. Generally, surgery may affect the protein binding of drugs *via* the presence of FFAs either in plasma or in tissues (23,38,39).

Furthermore, in Chinese subjects a decrease in AGP was observed resulting to reduction of the bound drug. This would be expected to have predictable pharmacokinetic consequences that may result in differences in responsiveness and should be an essential component of comparative studies in subjects of different races (18).

The decreased protein binding in various diseases is probably due to modifications of the albumin compartment volume and the presence of pathological inhibitors of drug binding on albumin binding sites (40-44).

Situations accompanied by decreases of proteins, such as starvation (vegetarian or religious fasting people), third age, prolonged bleeding or diseases affecting protein profile, are characterized by reduced total plasma proteins, or reversion of the plasma albumin/globulin fraction (as in disturbed connective tissue physiology) that lead to limited protein binding.

The administration of different NSAIDs in an experimental Freud's adjuvant induced arthritis model with demonstrated an enhancement of free drug concentration due to the decreased albumin level and to the inversion of albumin /globulin fraction (40).

Furthermore when a binding site e.g. site I is saturated by a compound, its excess amount can be bound to another site. When ampicillin is simultaneously given with chloropromazine, an enhancement of the ampicillin antimicrobial activity occurs. Although chlophenothiazine has a basic pKa (7.8) and ampicillin an acidic one (3.5), it is possible that a competition for another albumin binding site, apart from the common site I for acidic drugs, occurs. This may be

attributed to the increase of its free fraction in serum, or to a delayed renal elimination. It must be noted that chlorpromazine is mainly hepatically eliminated but a portion is excreted through the kidneys (45).

The simultaneous treatment of laboratory animals or patients with lidocaine and propranolol leads to an increase of lidocaine and eventually to a toxic effect on the heart muscle (46).

Liver injuries performed by CCl_4 in rats treated with the cationic lidocaine or propranolol resulted to the enhancement of lidocaine and propranolol serum levels and consequently to a pronounced effectiveness (47,48).

On the other hand in renal failure, reduced fractions of many acidic drugs bound to serum albumin are observed. (41). When the margin between therapeutic and toxic doses is narrow, drug monitoring by measurement of plasma free drug concentrations seems necessary to adjust the dosage regimen in uraemic patients (11,15,49).

The pharmacological effect and the rate of elimination by biotransformation or excretion are related to free drug concentration present in serum but particularly in tissues. The increased binding to tissue protein accelerates indirectly (secondary) the rate of elimination, since more drug is present on the metabolic site, but simultaneously the larger the free fraction the greater the elimination rate and the lower the total remaining amount. The concentration of a drug in plasma or its binding to plasma albumin does not represent the whole drug disposition or the changes occurring during the drug kinetics in the body (52).

The drug, once circulated in plasma, it can be distributed simultaneously in the interstitial fluid, in the cell and the other body fluids. In many pharmacokinetic models, the drug binding to albumin is linear. Albumin apart from the blood, is located in high percentage in the extravascular compartment e.g. the skin has an albumin content of 18% while the muscle about 15%. About 60% of the total albumin and 40% of AGP are localized in the interstitial space (51,52).

TISSUE BINDING OF DRUGS

A number of other binding materials apart from albumin interfere with the binding of drugs to various tissues. The influence of binding in tissues depends on the magnitude of each tissue volume, e.g. muscle constitutes about 40% of body mass, then tissue binding can be determined by muscle binding. Moreover, it can be suggested that muscle binding may be at least as important as plasma binding (52).

In the tissues, it is identified that an important anion, the ligandin, is the binding protein interacting with corticosteroid binder I and Y protein. Ligandin has a molecular weight of 42 KD and constitute 4% of the rat liver protein, but appreciable amounts are found in the tubuli cells of the kidney and the mucosal cells of the small intestine. Ligandin binds with endogenous and exogenous substances noncovalently with various binding affinities (53).

The extent of binding to plasma and to tissue-homogenates *in vitro*, when determined theoretically, demonstrated similarities with the in vivo findings of tissue-plasma partition ratios for anionic drugs. However, marked differences were observed between measured and predicted tissue-plasma ratios of lipophilic cationic drugs (55).

Drug binding to lipid-depleted tissues differ only to a moderate extent in comparison to control, suggesting, that tissue lipids may play a marginal role on drug binding, the principal role remaining to proteins (52). Most infections localised on the extravascular area in certain organs or systems and the success of antimicrobial treatment depends on the concentration of the chemotherapeutic agent on the target tissue or organ. A series of studies have reported similar findings in the extravascular area with those observed in serum. In addition, drug interactions, such as drug displacement (e.g. antimicrobials displaced by NSAIDs or lidocaine by propranolol) from albumin show similarities in many tissues, in normal or disease states. Possibly the occurring in vivo complexities may be due to interactions between drugs and endogenous ligands for HSA or tissue proteins (40,54,56).

Changes in protein binding may alter substantially the relationship between plasma concentration of total drug and the magnitude of pharmacological effect. The model of drug-plasma albumin binding shows similarities with the binding capacity of tissue proteins. In liver, a high non-specific binding of various NSAIDs (oxyphenbutazone, ibuprofen, ketoprofen, flurbiprofen and acetyl salicylic acid) has been observed to displace cationic or basic substances like warfarin and clonidin from its binding site *in vitro* (57).

It can be mentioned that a decrease in the extent of drug plasma protein binding does not necessarily lead to enhanced drug effects and therefore raises two important questions.

Firstly, does reduced protein binding have a clinically significant influence on the pharmacological effects of the drug? Secondly is it preferable to modify the dosage regimen of the drug or to correct the

plasma protein concentration prior to the administration of the drug?

At present, only tentative answers can be given (1) and it is not possible always to define or to correct the protein plasma concentration. Despite the limitations in the assessment of drug binding to proteins, the pharmacokinetic monitoring may be an important approach for the qualitative estimation of changes in tissue and plasma, during the course of the disease.

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