

MOLECULAR AND FUNCTIONAL STUDIES OF DRUG-PLASMA PROTEIN INTERACTIONS

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Drugs are distributed in the circulation either freely dissolved in the aqueous phase of the plasma, or bound with plasma proteins forming reversible drug-protein complexes. Hence, protein binding of drugs has a great impact on the pharmacokinetics and pharmacological effects of drugs. It is one of the most important areas of investigation in drug pharmacokinetics research. Drug binding to plasma proteins was first considered to be a nonspecific physical phenomenon, similar to the adsorption of small molecules to charcoal. Accumulated evidence indicate that drug binding to albumin and α_1 -acid glycoprotein (AGP) at low drug/protein molar ratios occurs at few specific and discrete ligand binding sites of both proteins. Therefore, information on the properties of these drug binding sites is important for understanding pharmacokinetically relevant binding phenomena such as displacement between different drugs and the dramatically altered plasma protein binding of some drugs during several disease states as well.¹⁾ In this conference, I

will talk about recent findings related to the mapping of drug binding sites on HSA, drug-plasma protein binding in certain disease states, the possible clinical utility of HSA, and the drug-binding properties of AGP. Topology analysis of drug binding sites on HSA and AGP determined using various methods, including spectroscopy, QSAR, photoaffinity labeling, site directed mutagenesis and X-ray crystallography will be discussed. In the crystal structures of HSA and recombinant HSA (rHSA), site I is a pocket in subdomain IIA. Some amino acid residues including 242His, 222Arg and 214Trp contribute to the site I ligand bindings. Crystallographic analyses have disclosed the location of site II to be in subdomain IIIA. 391Asn, 410Arg and 411Tyr are important for the binding of site II ligands. Recombinant albumins with point mutations were found to be useful for rapid identification of drug binding sites (Fig. 1).

In contrast to HSA, there seems to be no discrete binding sites on AGP, but drug binding occurs at a site with subregions. Trp, Tyr and His residues, especially His97 and Trp120 in the drug binding pockets play important roles in this process. Drug displacement is somewhat complex, due to the involvement of multiple factors. The significant inhibition in serum binding of drugs observed in uremic patients may be explained by a mechanism that involves a combination of direct displacement by fatty acids as well as cascade effects of fatty acids and uremic toxins. Albumin-containing dialysate is useful for the extracorporeal removal of endogenous toxins and in the treatment of drug overdoses. Oxidized albumin is a useful biomarker for the quantitative and qualitative evaluation of oxidative stress. Interestingly, AGP undergoes a structural transition to a unique structure (β -sheet to α -helix transformation) that differs from those of the native and denatured states, when it interacts with membranes.

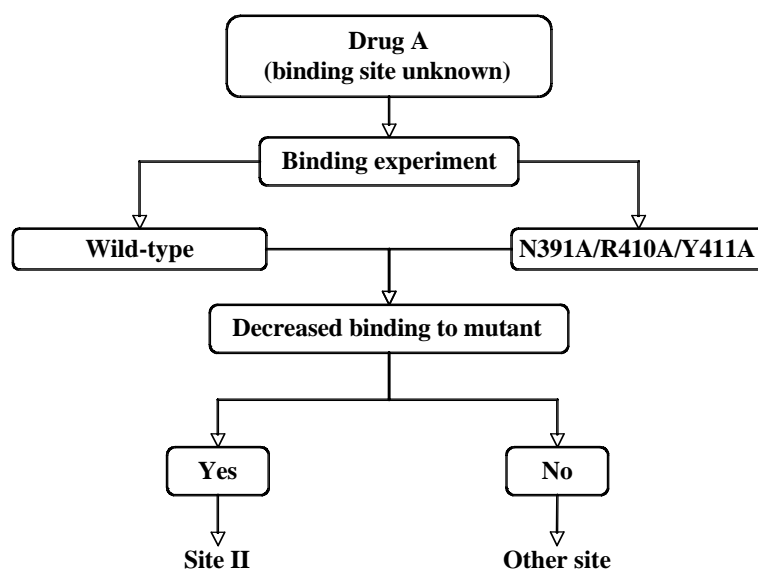


Fig.1

New screening method for identifying the location of a primary drug binding site of HSA using site II as an illustrative example.

References:

- 1) Kragh-Hansen, U., Chuang, V. T. G., and Otagiri, M.: Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. *Biol. Pharm. Bull.*, **21**: 695-704 (2002).