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Review

Clinical impact of serum proteins on drug delivery

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ABSTRACT

Among serum proteins albumin and transferrin have attracted the most interest as drug carriers in the past two decades. Prior to that, their potential use was overshadowed by the advent of monoclonal antibodies that was initiated by Milstein and Koehler in 1975. Meanwhile intensive pursuit of exploiting transferrin, but above all albumin as an exogenous or endogenous carrier protein for treating various diseases, primarily cancer, rheumatoid arthritis, diabetes and hepatitis has resulted in several marketed products and numerous clinical trials. While the use of transferrin has clinically been primarily restricted to immunotoxins, albumin-based drug delivery systems ranging from albumin drug nanoparticles, albumin fusion protein, prodrugs and peptide derivatives that bind covalently to albumin as well as physically binding antibody fragments and therapeutically active peptides are in advanced clinical trials or approved products. For treating diabetes, Levemir® and Victoza® that are myristic acid derivatives of human insulin or glucagon-like peptide 1 (GLP-1) act as long-acting peptides by binding to the fatty acid binding sites on circulating albumin to control glucose levels. Levemir® from Novo Nordisk has already developed into a blockbuster since its market approval in 2004. Abraxane®, an albumin paclitaxel nanoparticle as a water-soluble galenic formulation avoiding the use of cremophor/ethanol, transports paclitaxel through passive targeting as an albumin paclitaxel complex to the tumor site and is superior to conventional Taxol® against metastatic breast cancer. INNO-206, an albumin-binding doxorubicin prodrug that also accumulates in solid tumors due to the enhanced permeability and retention (EPR) effect but releases the parent drug through acid cleavage, either intra- or extracellularly, is entering phase II studies against sarcoma. An expanding field is the use of albumin-binding antibody moieties which do not contain the fragment crystallizable (Fc) portion of, conventional immunoglobulin G (IgG) but are comprised of monovalent or bivalent light and/or heavy chains and incorporate an additional albumin-binding peptide or antibody domain. The most advanced antibody of this kind is ATN-103 (Ozoralizumab), a trivalent albumin-binding nanobody that neutralizes the pro-inflammatory tumor necrosis factor alpha (TNF-α) as a causative agent for exacerbating rheumatoid arthritis. ATN-103 is currently in multi-center phase II trials against this debilitating disease. In summary, because albumin as the most abundant circulating protein cannot only be used to improve the pharmacokinetic profile of therapeutically relevant peptides and the targeting moiety of antibodies but also for peptide-based targeting as well as low-molecular weight drugs to inflamed or malignant tissue, it is anticipated that R&D efforts of academia and the pharmaceutical industry in this field of drug delivery will prosper.

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1. Introduction

Any drug, whether applied orally, intravenously, sublingual, subcutaneous or intramuscularly, is transported by the blood and its first encounter is not only with the various cellular components and low-molecular weight compounds, but also with a multitude of plasma proteins. The complexity of the human plasma proteome is immense comprised of approximately 100,000 proteins whose concentrations span over 12 orders of magnitude. The major components can be separated by conventional cellulose acetate or PAGE electrophoresis and are shown in Fig. 1.

Albumin is by far the most abundant protein with a concentration of ~35–50 mg/mL. The amounts of other major plasma proteins are much lower (see Fig. 1), e.g. with concentrations of transferrin in the range of ~2.5–3.5 mg/mL. Further separation and identification of all plasma proteins and peptides is a formidable task, and indeed the high abundant plasma protein components (primarily albumin and immunoglobulins) have to be removed prior to the identification of plasma proteins by 2D gel electrophoresis in the low nano- or picomolar range followed by subsequent 2D electrophoresis. In this way approximately 600–1000 peptides and proteins can be separated (see for example http://www.biocompare.com).

Drugs can bind to several blood components, such as albumin, α_1-acid glycoprotein, lipoproteins and immunoglobulins, but due to the large excess and small size of albumin this protein is predominantly involved. The extent of plasma protein binding for basically all drugs is stated on index of medicines or patient instruction leaflets and is generally determined by equilibrium dialysis with the protein-fraction bound corresponding to the same or similar value if pure human serum albumin is used. Intuitively, this value is taken as being predictive for the pharmacokinetic characteristics of the drugs in question by drug developers and clinicians and is also taken into account when analyzing drug-drug interactions and disease drug interactions leading to increased free plasma levels with concomitant drug exposure and unforeseen side effects or loss of activity. However, this can be considered as a general misconception, and changes in plasma protein binding have little clinical relevance for most drugs [1, 2].

The reasons – although the unbound drug is regarded as the therapeutically active fraction that exerts its effect on the molecular target within cells and tissues – are threefold: (1) The half-life, clearance and tissue distribution of the vast majority of low-molecular weight drugs are so fast (in the order of minutes or at the most a few hours) that the fraction of the drug that is actually protein-bound in the plasma is very small or sometimes negligible; (2) The binding constants of the drug for albumin are generally low ($Kd < 10^6$ M) so the drug displays a very rapid pharmacokinetic-pharmacodynamic equilibration time; (3) As a consequence, protein-binding of a drug measured in an isolated non-dynamic system outside of the body can but often does not correlate with other pharmacokinetic parameters such as plasma half-life, clearance, area under the curve (AUC), or volume of distribution. We have no intention to propagate that drug-drug interactions be ignored, these obviously are present and certainly belong to an important field of research of their own, but protein-binding as routinely determined and stated for commonly prescribed drugs is a poor predictor for the displacement of drugs from their protein-bound state and rarely alters a patient's overall exposure to a drug [1]. Indeed, renal or hepatic elimination or the metabolism of the drug may influence the toxicity profile of a drug to a much larger extent than the small fraction that is protein-bound.

When moving to the area of drug delivery, plasma protein-binding becomes important when the binding constants are higher, in the order of 10^7–10^9 M, because in these cases a major fraction of the administered drug is bound to plasma proteins, primarily albumin. This is true for endogenous ligands such as fatty acids or bilirubin which both bind tightly to albumin and are transported in the albumin-bound form in the body with the fraction of free ligand being less important. Scientists in drug design have used these insights to develop drugs with high binding affinity for albumin to either improve the pharmacokinetic profile and bioavailability of peptides and antibody moieties or to exploit the targeting property of albumin for inflamed or malignant tissue.

Although human serum albumin (66.5 kDa) is a transport protein per se trafficking fatty acids, metal ions (Ca^{2+}, Zn^{2+}, Cu^{2+}), bilirubin and high binding drugs within the body, historically other transport proteins such as transferrin and the low density lipoprotein (LDL) as a source of supplying the cells with iron (III) and cholesterol, respectively, in the body first attracted the attention of scientists involved in drug delivery or diagnostic applications.

Transferrin (78 kDa) is responsible for the transfer of iron(III) and ferries it between very different cell types such as the intestine epithelium, where iron enters the body from the diet, the liver, where it is stored as ferritin (a protein containing iron–phosphate–hydroxide complexes), the developing erythroid cells, which require it for hemoglobin synthesis, and cells which need iron for cell growth including tumor cells. LDL is the principal carrier of cholesterol to tissues.

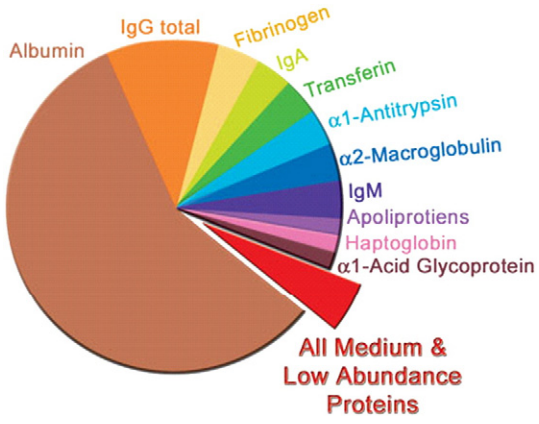

Fig. 1. Overview of the major human plasma proteins that can be identified by simple electrophoretic techniques.