Pharmaceutical biotechnology

Lecture-8

Distribution of protein therapeutics

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Distribution Mechanisms and Volumes

- The rate and extent of protein distribution is determined largely by

1. Their size and molecular weight
2. Physicochemical properties (e.g., charge, lipophilicity)
3. Protein binding, and
4. Their dependency on active transport processes.

- Since most therapeutic proteins have high molecular weights and are thus large in size, their apparent volume of distribution is usually small and limited to the volume of extracellular space due to their limited mobility secondary to impaired passage through biomembranes
- Active tissue uptake and binding to intra- and extravascular proteins, however, can substantially increase the apparent volume of distribution of protein drugs. (For example volume of distribution of interferon β-Ib is 2.8 L/ Kg)

- In contrast to small molecule drugs, protein transport from vascular space into the interstitial space of tissues is largely mediated by convection rather than diffusion, following the unidirectional fluid flux from the vascular space through paracellular pores into the interstitial tissue space.
- The subsequent removal from the tissues is accomplished by lymph drainage back into the systemic circulation.
- Another, but much less prominent pathway for the movement of protein molecules from the vascular to the interstitial space is transcellular migration via endocytosis.

- Besides the size-dependent sieving of macromolecules through the capillary walls, charge may also play an important role in the biodistribution of proteins.

✓ It has been suggested that the electrostatic attraction between positively charged proteins and negatively charged cell membranes might increase the rate and extent of tissue distribution.

- Most cell surfaces are negatively charged because of their abundance of glycoaminoglycans in the extracellular matrix.

Pharmacokinetics of proteins

- After IV administration, peptides and proteins usually follow a biexponential plasma concentration-time profile that can best be described by a two-compartment pharmacokinetic model.
- The central compartment in this two-compartment model represents primarily the vascular space and the interstitial space of well-perfused organs with permeable capillary walls, including the liver and kidney.
- The peripheral compartment is more reflective of concentration-time profile in the interstitial space of slowly equilibrating tissues.

- The central compartment in which proteins initially distribute after IV has thus typically a volume of distribution equal or slightly larger than the plasma volume, i.e., 3 to 8 L.
- The total volume of distribution frequently comprises with 14 to 20 L not more than 2 to 3 times the initial volume of distribution.

- An example for such a distribution pattern is the t-PA analog tenecteplase.

➢ Radiolabeled ^{125}I-tenecteplase was described to have an initial volume of distribution of 6.1 to 9.9 L with liver as the only organ that had a significant uptake of radioactivity.

✓ The authors concluded that the small volume of distribution suggests primarily intravascular distribution for tenecteplase, consistent with the drug's large molecular weight of 65 kDa.

Determination volume of distribution for proteins

- The pharmacokinetic calculations of volume of distribution may be problematic for many protein therapeutics.
- Non-compartmental determination of volume of distribution at steady stat (V_{SS}) using statistical moment theory assume first-order disposition processes with elimination occurring from the rapidly equilibrating or central compartment.

- These basic assumptions, however, are not fulfilled for numerous protein therapeutics, as proteolysis and receptor-mediated elimination in peripheral tissues may constitute a substantial fraction of the overall elimination process.
- If protein therapeutics are eliminated from slowly equilibrating tissues at a rate greater than their distribution process, substantial error in the volume of distribution assessment may occur.

- A recent simulation study could show that if substantial tissue elimination exists, a V_{ss} determined by non-compartmental methods will underestimate the "true" V_{ss}, and that magnitude of error tends to be larger the more extensively the protein is eliminated by tissues routes.

- These challenges in characterizing the distribution of protein therapeutics can only be overcome by determining actual protein concentrations in the tissue by biopsy or necropsy, or via biodistribution studies with radiolabeled compound and / or imaging techniques.

- Biodistribution studies are imperative for small organic synthetic drugs, since long residence times of the radioactive label in certain tissues may be an indication of tissue accumulation of potentially toxic metabolites.
- Because of the possible reutilization of amino acids from protein drugs in endogenous proteins, such a safety issue does not exist for protein therapeutics.

- Therefore, biodistribution studies for protein drugs are usually only performed to assess drug targeting to specific tissues, or to detect the major organs of elimination (usually kidneys and liver).
- If the protein contains a suitable amino acid such as tyrosine or lysine, an external label such as 125I can be chemically coupled to the protein.
- Although this coupling is easily accomplished and a highly specific activity can be obtained, the protein is chemically altered.
- Therefore, it may be better to label proteins and other biotechnology compounds by introducing radioactive isotopes during their synthesis by which an internal atom becomes the radioactive marker (internal labeling).

- For recombinant proteins, internal labeling can be accomplished by growing the production cell line in the presence of amino acids labeled with 3H, 14C, 35S, etc.
- This method is not routinely used because of the prohibition of radioactive contamination of fermentation equipment.
- Moreover, internally labeled proteins may be less desirable than iodinated proteins because of the potential reutilization of the radiolabeled amino acid fragments in the synthesis of endogenous proteins and cell structures.
- Irrespective of the labeling method, but more so for external labeling, the labeled product should have demonstrated physicochemical and biological properties identical to the unlabeled molecule.

- In addition, as for all types of radio labeled studies, it needs to be established whether the measured radio activity represents intact labeled protein, or radiolabeled metabolites, or the liberated label.
- Trichloro-acetic acid precipitable radioactivity is often used to distinguish intact protein from free label or low-molecular-weight metabolites, which appear in the supernatant after centrifugation.
- Proteins with reutilized labeled amino acids and large protein metabolites can only be distinguished from the original protein by techniques such as polyacrylamide gel electrophoresis (PAGE), high pressure liquid chromatography (HPLC), specific immunoassays, or bioassays.

- This discussion also implies that the results of biodistribution studies with autoradiography can be very misleading.
- Auto-radiography is a technique where tissue samples are brought into contact with X-ray sensitive films to visualize radioactively labeled molecules or fragments of molecules.
- Although auto-radiography is becoming more quantitative, one never knows what is being measured qualitatively (original molecules or its degradation products) without specific assays.
- It is therefore sometimes better to perform biodistribution studies by the collection of the tissues and the specific measurement of the protein drug in the tissue homogenate.

Protein Binding of Protein Therapeutics

- Another factor that can influence the distribution of therapeutic peptides and proteins is binding to endogenous protein structures.
- Physiologically active endogenous peptides and proteins frequently interact with specific binding proteins involved in their transport and regulation.
- Furthermore, interaction with binding proteins may enable or facilitate cellular uptake processes and thus affect the drug's pharmacodynamics.
- Similarly, therapeutically administered proteins may interact with endogenous binding proteins.

- It is a general pharmacokinetic principle, which is also applicable to proteins, that only the free, unbound fraction of a drug substance is accessible to distribution and elimination processes as well as interaction with its target structures at the site of action, for example a receptor or ion channel.

➢ Thus protein binding may affect the pharmacodynamics, but also disposition properties of protein therapeutics.

- Specific binding proteins have been identified for numerous protein drugs, including recombinant human DNase for use as mucolytic in cystic fibrosis, growth hormone, and recombinant human vascular endothelial growth factor (rhVEGF).

- Protein binding not only affects the unbound fraction of a protein drug and thus the fraction available to exert pharmacological activity, but many times it also either prolongs protein circulation time by acting as a storage depot or it enhances protein clearance.

Examples

- Recombinant cytokines, for example, may after IV administration encounter cytokine-binding proteins including soluble cytokine receptors and anti-cytokine antibodies.
- In either case, the binding protein may either prolong the cytokine circulation time by acting as a storage depot or it may enhance the cytokine clearance.

Examples

- Growth hormone, as another example, has at least two binding protein in plasma.
- This protein binding substantially reduce growth hormone elimination with a tenfold smaller clearance of total compared to free growth hormone, but also decreases its activity via reduction of receptor interaction.

- Apart from these specific bindings, peptides and proteins may also be non-specifically bound to plasma proteins.
- For example, metkephamid, a metenkephalin analog, was described to be 44% to 49% bound to albumin, and octreotide, a somatostatin analog, is up to 65% bound to lipoproteins.

Distribution via Receptor-Mediated Uptake

- Aside from physicochemical properties and protein binding of protein therapeutics, site-specific receptor-mediated uptake can also substantially influence and contribute to the distribution of protein therapeutics, as well as to elimination and pharmacodynamics.
- The generally low volume of distribution should not necessarily be interpreted as low tissue penetration.
- Receptor-mediated specific uptake into the target organ, as one mechanism, can result in therapeutically effective tissue concentrations despite a relatively small volume of distribution.

Example

- Nartograstim, a recombinant derivative of the granulocyte-colony-stimulating factor (G-CSF), for example, is characterized by a specific, dose-dependent and saturable tissue uptake into the target organ bone marrow, presumably via receptor-mediated endocytosis.