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Distribution and elimination of protein therapeutics: A review

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Review Article

ABSTRACT

It was 1980s when the first therapeutic protein was launched in the market. It was recombinant DNA-derived insulin. Since its inception, within the worldwide pharmaceutical sector, protein therapeutics has been enjoying the fastest growth, notably for the last few years. As a result it is assumed that the treatment methodology with the conventional drug therapy will be shifted towards therapeutic proteins in near future. It made revolution in the treatment of chronic diseases like cancer, diabetes, cardiovascular diseases. The major segments in protein therapeutics are monoclonal antibody, insulin, granulocyte-colony stimulating factor (G-CSF), coagulation factors etc. In this review paper we will discuss the general aspects of protein therapeutics with their advantages over small-molecule drugs, functional classification of therapeutic proteins and their uses. The pharmacokinetics of protein therapeutics, especially from the distribution and elimination characteristics of therapeutic proteins will be discussed in brief with relevant examples. The major challenges and future perspectives will also be presented in short.

Keywords: Therapeutic protein, Monoclonal antibody, Coagulation factors, Growth hormones, Insulin.

INTRODUCTION

Proteins have the most dynamic and diverse role of any macromolecule in the body, catalyzing biochemical reactions, forming receptors and channels in membranes, providing intracellular and extracellular scaffolding support, and transporting molecules within a cell or from one organ to another (Leader et al., 2008). In a review paper by Reichert (2003) it was showed that the average clinical development and approval time was more than 1 year faster for 33 protein therapeutics approved between 1980 and 2002 than for 294 small-molecule drugs approved during the same time period. Once a rarely used subset of medical treatments, protein therapeutics have increased dramatically in number and frequency of use since the introduction of the first recombinant protein therapeutic human insulin- 25 years ago. More than 130 proteins (over 95 of which are produced recombinantly) are currently approved for clinical use by the FDA, and many more are in development (Leader et al., 2008). Nowadays protein therapeutics is one of the most important as well as vastly studied areas in medicine. It was estimated earlier from the market opportunity point of view that the global protein therapeutics market will be worth $77 billion by 2011 with biogenerics playing an increasingly important role (ActiVery, 2011).

Protein therapeutics included mostly recombinant versions of naturally occurring proteins (Caravella and Lugovskoy, 2010; Leader et al., 2008) and F_c fusion proteins and antibodies which are not naturally occurring human proteins (Caravella and Lugovskoy, 2010). Therapeutic proteins are usually defined as the proteins that have an effect of healing or use inside biological system and play diversified roles (Mukherjee,

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Table 1. Different biological roles of therapeutic proteins.

Protein	Therapeutic role
Albumin	Nutrition
Gamma globulins	Boosts body defenses against infectious diseases
Synthetic	Antibodies against inflammatory proteins components (infliximab), or against tumor components (trastuzumab)

2011) in the body as shown in Table 1.

According to a definition given by European Medicines Agency, therapeutic proteins include different molecules ranging from peptides to large proteins such as coagulation factors (EMEA, 2007).

An increasing number of potential protein therapeutic agents move into preclinical and clinical development (Gloff and Benet, 1990) and thus the proper evaluation of pharmacokinetics of them; characterization of the time course of drug absorption, distribution, metabolism and excretion (Atkinson, 2001) becomes more critical. But for macromolecules, the evaluation of PK parameters can be complicated by several factors and must be considered before conducting a PK study (Mahmood, 2006).

Generally, the requirements for therapeutic proteins with respect to evaluating the pharmacokinetics of the product are the same as for conventional products, but specific considerations are needed related to the inherent characteristics of proteins. The pharmacokinetics should be characterized during single-dose and steady-state conditions in relevant populations. However, the pharmacokinetic requirements may differ depending on the type of protein and its intended use (EMEA, 2007).

In this review we took an attempt to define therapeutic proteins, with their types, advantages, major challenges of this class of drugs, explain their pharmacokinetic behavior from their distribution and elimination perspectives. A detailed tabular presentation of some common therapeutic proteins with their distribution and elimination characteristics has also been included in the review.

PROTEIN THERAPEUTICS: ENGINEERED PHARMACEUTICALS

The attractiveness of proteins as therapeutics stems in part from the exquisite specificity by which they execute diverse functions-e.g., they catalyze exactly the right reaction or inhibit exactly the right cell receptor (Wright et al., 2011). It is currently estimated that there are 25,000–40,000 different genes in the human genome and with alternative splicing of genes and post-translational modification of proteins (for example, by cleavage, phosphorylation, acylation and glycosylation), the number of functionally distinct proteins is likely to be much higher. Viewed from the perspective of disease mechanisms, these estimates pose an immense challenge to modern medicine (Leader et al., 2008).

Proteins that are engineered in the laboratory for pharmaceutical use are known as therapeutic proteins (LeadDiscovery, 2005). PROLOR Biotech Inc. defined therapeutic proteins as proteins that are either extracted from human cells or engineered in the laboratory for pharmaceutical use (PROLOR Biotech Inc., 2006).

ADVANTAGES OF PROTEIN THERAPEUTICS OVER SMALL-MOLECULE DRUGS

Therapeutic proteins have several advantages over small molecule drugs. The advantages may be summarized as bellow (Leader et al., 2008):

- Proteins are highly specific and perf orm complex set of function which may not be mimicked by simple chemical compounds.
- High specificity minimizes the chance of adverse effects.
- Therapeutic proteins are assumed to be well tolerated as body naturally produces many of them.
- Incase of genetic disorders, ef fective replacement treatment may be possible without the need of gene therapy.
- Clinical development an d FDA approval time of protein therapeutics may be faster than that of small-molecule drugs.
- Companies are able to obtain far-reaching

patent protection for protein therapeutics as proteins are unique in form and function.

The last two advantages make proteins attractive from a financial perspective compared with small-molecule drugs.

FUNCTIONAL CLASSIFICATION OF PROTEIN THERAPEUTICS

The functional classification of therapeutic proteins is given in Table 2.

Table 2: Functional classification of protein therapeutics (adopted from Leader et al., 2008).

Group I: protein therapeutics with enzymatic or regulatory activity	
Ia	Replacing a protein that is deficient or abnormal
Ib	Augmenting an existing pathway
Ic	Providing a novel function or activity
Group II: protein therapeutics with special targeting activity	
IIa	Interfering with a molecule or organism
IIb	Delivering other compounds or proteins
Group III: protein vaccines	
IIIa	Protecting against a deleterious foreign agent
IIIb	Treating an autoimmune disease
IIIc	Treating cancer
Group IV : protein diagnostics	

PHARMACOKINETICS OF PROTEIN THERAPEUTICS

Pharmacokinetic evaluation of therapeutic agents involves the determination of four critical parameters: clearance, volume of distribution, half-life and bioavailability (Gloff and Benet, 1990). For proteins, evaluation of these parameters is often complicated by a number of factors. The difficulties regarding this issue can be summarized as bellow (Mahmood and Green, 2005):

1. Difficulty in identifying and quantitating metabolites.

2. More extensive sites of metabolism within the body due to the ubiquitous nature of proteases.

3. The binding of the protein therapeutic to endogenous proteins.

4. Low absorption of intact molecules across biological membranes.

5. The assay methodologies currently available to measure concentration in body fluids.

6. Complications during the preclinical pharmacologic and toxicologic evaluation as some potential protein therapeutic agents also exhibit species specificity.

7. Many protein therapeutic agents, as compared to the native molecule, exhibit amino acid substitutions, additions/deletions of amino acids at the ends of the molecule and/or loss of carbohydrate attachments (glycosylation).

8. In many cases the mechanism and site of action are unknown for these compounds which make hard to determine what route, frequency or time of administration will be most efficacious.

9. The relationship between pharmacokinetics and pharmacodynamics of protein therapeutic agents is often unclear.

10. The interaction of protein drugs with other therapeutic moieties may be complicated by the ability of some proteins to increase or decrease receptor numbers and/or affinities.

Distribution and elimination of therapeutic proteins

Pharmacokinetic study of a drug includes, in simple form, the study of drug absorption, distribution, metabolism (biotransformation) and elimination. Due to gastrointestinal enzymatic degradation of the protein molecules, most of the therapeutic proteins are not administered via the oral route. In general the intravenous and subcutaneous routes of drug administration are generally preferred for therapeutic proteins. However, after reaching the bloodstream, a protein molecule is distributed intracellularly by

distributing itself through the vascular space, transporting across the microvascular wall, transporting through the interstitial space and transporting across cell membranes (Braeckman, 2000). It was found that in case of subcutaneous administration of proteins or protein like macromolecules, with increasing molecular weight of proteins the lymphatic system becomes the predominant pathway for drug absorption. Proteins with the molecular weight more than 16 kDa are mainly absorbed by lymphatic system (Supersaxo et al., 1988; Supersaxo et al., 1990).

Distribution

The intracellular biodistribution of the macromolecules depends on several factors (Mahmood and Green, 2005), i.e.

i) the physicochemical properties of the molecule

ii) physicochemical properties of the capillaries involved in the process

iii) structure of the capillaries responsible for the passage of the molecule from systemic circulation to the intestinal fluid

iv) the presence of the receptors

For therapeutic proteins, which are administered subcutaneously a unique transport mechanism was reported to follow during the transport of proteins from the lymphatic system to the systemic circulation (Mahmood and Green, 2005). plasma binding proteins were also reported to serve as transporters and activators, especially for those drugs that pass membranes by active processes (Maack, 1975).

Elimination

Clearance of protein drugs from the systemic circulation begins with passage across the capillary endothelia (Kompella and Lee, 1991). This endothelial passage depends on two factors (presented at Table 3).

Elimination of protein drugs involves: renal excretion and hepatic elimination.

Renal elimination

The overall renal elimination of protein drugs can be summarized by the Table 4.

Table 3: Factors related to the clearance of protein drugs via endothelial passage.

Factor related to protein molecules	Factors related to capillaries
Size	Structural properties
Shape	Physicochemical
Charge	properties

After glomerular filtration the protein or peptide may face three possible fates, i) can be excreted unchanged in the urine, ii) may be degraded to such compounds those are excreted in the urine, iii) active reabsorption may occur by the proximal tubules by a process known as luminal endocytosis. The third possible fate is shown by a conceptual diagram in Figure 1.

Hepatic Elimination

Liver plays an important role in the removal of proteins from the systemic circulation. Several mechanisms have been suggested for the hepatic elimination of protein drugs; i.e. RME – Receptor mediated endocytosis, non-selective pinocytosis (Kompella and Lee, 1991; Bocci, 1990), receptors-mediated uptake (Braeckman, 2000).

Biliary elimination

Some proteins can also be cleared from the systemic circulation by biliary excretion. Some proteins enter the bile from plasma across hepatocytes via specific transport process. Insulin and epidermal growth factor are examples of therapeutic proteins that are excreted in the bile (Preusch, 2001).

Table 5 represents examples of some therapeutic proteins with available brand name, pharmacokinetic parameter in relation to distribution and elimination.

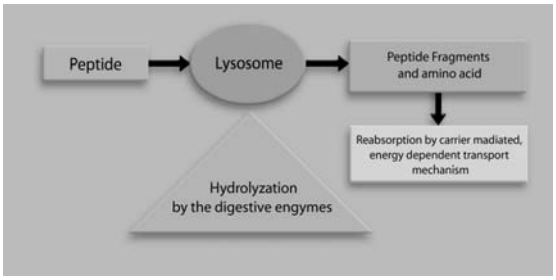

Figure 1: Schematic diagram of active reabsorption of protein drugs in kidney. Conceptually constructed from Mahmood and Green, 2005.

Table 4: Renal elimination of therapeutic proteins.

Factor	Specification	Remarks	Reference
Molecular weight of proteins	Less than 30 kDa	Filtered by the glomerulus	Kompella and Lee, 1991
	Less than 5 kDa	Undergo efficient normal glomerular filtration with a normal GFR of 120 ml/min	Maack et al., 1979
	More than 30 kDa	The capacity of a protein for glomerular filtration decreases	Kompella and Lee, 1991
Charge and size of proteins	Anionic molecules	As the glomerular filter is negatively charged the anionic molecules (INF alpha, INF beta and TNF alpha) are repelled and not filtered	Bocci, 1990

CURRENT CHALLENGES AND FUTURE PERSPECTIVES IN PROTEIN THERAPEUTICS

Proteins have several significant limitations as therapeutics. For example, protein therapeutics are very expensive, reflecting high production costs, that may limit patient access and also clinical applications. Intracellular delivery of proteins is possible in the laboratory setting, e.g., by protein transduction.

Oral bioavailability is another short coming of protein therapeutics. All therapeutic proteins are potentially immunogenic in patients and occasionally an anti-drug antibody response can lead to a major safety issue.

Overall, there are enormous challenges involved in the development of therapeutic proteins (Mahmood and Green, 2005), such as:

i. The current available analytical methods are unable to distinguish between bioactive and non-active components of many therapeutic proteins.

ii. Evaluation of the metabolism of therapeutic proteins is complicated, and is generally not done.

iii. The binding of therapeutic proteins to endogenous proteins has been largely ignored.

iv. The formation of antibodies in response to the administration of a therapeutic protein can alter the pharmacokinetics and pharmacodynamics of protein drugs.

v. Minor changes in the structure of a protein (glycosylation or pegylation) can alter its pharmacokinetics and pharmacological response.

vi. The relationship between pharmaco-kinetics and pharmacological response (pharmacodynamics) of therapeutic proteins is, in most cases, unclear due to a lack of understanding of the mechanism and site of action of the compound.

vii. Species specificity in pharmacological response can complicate the preclinical evaluation of therapeutic proteins.

viii. The route, site and time of administration of macromolecules can have an impact on the pharmacokinetics and pharmacological response of therapeutic proteins.

But it is thought that commercially protein therapeutics may be a success. Clinical and commercial success with protein therapeutics has bred intense competition between different organizations striving to develop protein therapeutics to the same antigen and/or overlapping therapeutic indications. As

Table 5: Examples of protein therapeutics with their distribution and elimination characteristics

Therapeutic protein (Brand name)	Distribution	Elimination	Reference
Insulin (Novolin)	Plasma protein binding 0-9%	Rapidly eliminated (half life 81 minutes)	GlobalRPh Inc., 2011
Pramlintide acetate (Symlin)	Approximately 40% of the drug is unbound in plasma	No bioaccumulation occurs. Half life 48 minutes	Symlin Product Literature, 2008
Growth hormone (GH), somatotropin (Serostim)	The steady-state volume of distribution (Mean ± SD) following IV administration of somatropin in healthy volunteers is 12.0 ± 1.08 L	Half life 4.28 hours. The renal clearance of r-hGH after subcutaneous administration in nine patients with AIDS related wasting was 0.0015 ± 0.0037 L/h	Serostim Product Monograph, 2007
Factor VIII (Bioclate)	Apparent volume of distribution at steady state 0.045-0.051 L/kg	Average elimination half-life is around 14 hours	Schwartz et al., 1990 Björkman and Berntorp, 2001
Factor IX (Benefix)	Apparent volume of distribution at steady state 0.22 L/kg	Average elimination half-life is around 19 hours,	Schwartz et al., 1990
Antithrombin III (AT-III) (Thrombate III)	Distribution half-life approximately 3 hours. Distributed into plasma (39%), extravascular space (49%), and vascular endothelial cells (11%)	Elimination half-life approximately 42 hours. Cleared principally by liver and excreted in urine	Cada et al., 2009, Health Encyclopedia, 2011
Protein C concentrate (Ceprotin)	Volume of distribution at steady state 0.70 to 0.89 dL/kg.	Initial half life 5.4 to 9.3 hours, whereas terminal half life 7.0 to 12.4 hours	FDA, 2007
Beta-Glucocerebrosidase (Cerezyme)	The volume of distribution corrected for weight ranged from 0.09 to 0.15 L/kg (0.12 ± 0.02 L/kg).	Half life 3.6 to 10.4 minutes. Plasma clearance ranged from 9.8 to 20.3 mL/min/kg (mean ± S.D., 14.5 ± 4.0 mL/min/kg).	Cerezyme Produc Insert
Alglucosidase-(Myozyme)	The majority of the material distributed to the liver with smaller amounts distributing to other organs (heart, spleen, lung and kidney). Only a small amount of material was measured in the skeletal muscles collected. Volume of distribution 53.8 ± 10.7 mL/kg	Half life 2.59 ± 0.23 hours. Clearance 13.9 ± 2.3 mL/hr/kg	EMEA, 2006a, Myozyme Product Information

Galsulphase (Naglazyme)	rhASB was widely distributed into tissues, with the largest proportion localised to the liver in all studies. There were also significant levels in the spleen, lung, kidney, heart, skin, aorta, cerebrum, cerebellum and lymph nodes	rhASB is cleared rapidly from the plasma, with a half-life of about 15 minutes following an intravenous dose of 1 mg/kg. As mentioned previously, the tissue half-lives are approximately 2 to 4 days at 1 mg/kg	EMEA, 2006b
Pooled immunoglobulins (Octagam)	After infusion, exogenous IgG is distributed relatively rapidly between plasma and extra-vascular fluid until approximately half is partitioned in the extravascular space	Studies show that the apparent half-life of is approximately 40 days in immunodeficient patients	Octagam Product Insert
Erythropoietin, Epoetin-(Procrit) -	-	In adult and pediatric patients with CRF, the elimination half-life of plasma erythropoietin after intravenously administered Procrit ranges from 4 to 13 hours.	Procrit Label
Darbepoetin-(Aranesp)	Volume of distribution 52.4 ± 2.0 (mL/kg)	Clearance 1.6 ± 0.3 mL/h per kg. Terminal half-life 25.3 ± 2.2 hr	Powell and Gurk-Turner, 2002
Pegfilgrastim (Peg-G-CSF) (Neulasta) -		The pharmacokinetics of pegfilgrastim were nonlinear and clearance decreased with increases in dose. Neutrophil receptor binding is an important component of the clearance of pegfilgrastim, and serum clearance is directly related to the number of neutrophils. The half-life of Neulasta ranged from 15 to 80 hours after subcutaneous injection.	Neulasta Prescribing Information
Oprelvekin (interleukin11; IL11) (Neumega)	The absolute bioavailability of Neumega was >80% in a study. Neumega did not accumulate and clearance of Neumega was not impaired following multiple doses.	Terminal half-life 6.9 ± 1.7 hrs. The kidney is the primary route of elimination.	Neumega Drug Insert

Human follicle-stimulating hormone (FSH) (Follistim)	The volume of distribution of Follistim in healthy, pituitary-suppressed, women following intravenous administration of a 300 international units dose was approximately 8 L.	Half life 33.4 hours. Clearance 0.01 L/h/kg	Drugs.com-Online resource on Follistim AQ
Lutropin- (Luveris)	A rapid distribution phase (t ½ 1 of approximately 1 hour) and a terminal half-life (t½) of approximately 11 hours were observed for r-hLH. The steady state volume of distribution (Vss) was approximately 10 L. Mean residence time (MRT) was approximately 6 hours	r-hLH is eliminated from the body with a mean terminal half-life of about 18 hours. Total body clearance is approximately 2 to 3 L/h with less than 5 percent of the dose being excreted unchanged renally.	Archived Drug Label of Luveris 2005
Urokinase (Abbokinase)	Distribution volume 11.5 L	Urokinase intravenous infusion is rapidly cleared by the liver with an elimination half- life for biologic activity of 12.6 +/- 6.2 minutes. Small fractions of the administered dose are excreted in bile and urine.	Abbokinase Approval Application, FDA 2002
Salmon calcitonin (Fortical)	Peak plasma concentrations of drug appear approximately 10 minutes after nasal administration.	The terminal half-life (t1/2) of calcitonin-salmon is calculated to be about 23 minutes. There is no accumulation of the drug on repeated nasal administration at 10 hour intervals for up to 15 days.	Drugs.com-Online resource on Fortical
Lepirudin (Refludan)	The pharmacokinetic properties of lepirudin following intravenous administration are well described by a two-compartment model. Distribution is essentially confined to extracellular fluids and is characterized by an initial half-life of approximately 10 minutes	Elimination follows a first-order process and is characterized by a terminal half-life of about 1.3 hours in young healthy volunteers. The systemic clearance of lepirudin is proportional to the glomerular filtration rate or creatinine clearance.	Drugs.com-Online resource on Refludan

Bevacizumab (Avastin)	The typical value for central volume (Vc) was 2.73 L and 3.28 L for female and male patients respectively, which is in the range that has been described for IgGs and other monoclonal antibodies. The typical value for peripheral volume (Vp) was 1.69 L and 2.35 L for female and male patients respectively	The value for clearance is, on average, equal to 0.188 and 0.220 L/day for female and male patients, respectively. According to the two-compartmental model, the elimination half-life is 18 days for a typical female patient and 20 days for a typical male patient	Data Sheet of Avastin
Cetuximab (Erbitux)	The volume of the distribution for cetuximab appeared to be independent of dose and approximated the vascular space of 2-3 L/m2.	The mean half-life of cetuximab was approximately 112 hours (range 63 -230 hours)	Erbitux Package Inserts
Rituximab (Rituxan)	Binds to lymphoid cells in thymus, white pulp of spleen, and a majority of B lymphocytes in peripheral blood and lymph nodes. In patients with rheumatoid arthritis (RA), the Vd was 3.1 L; in patients with Wegener granulomatosis or microscopic polyangiitis, the Vd was 4.5 L	The wide range of half-lives reflects the variable tumor burden and changes in CD19-positive, B-cell populations. In patients with RA, the Cl was 0.335 L/day and the mean half-life was 18 days. In patients with Wegener granulomatosis and microscopic polyangiitis, the Cl was 0.312 L/day and the half-life was 23 days. In patients with non-Hodgkin lymphoma (NHL) or chronic lymphocytic leukemia (CLL), the half-life was 22 and 32 days, respectively	Drugs.com-Online resource on Rituxan
Infliximab (Remicade)	Distribution into body tissues and fluids, including joints, has not been fully characterized. Not known whether infliximab crosses the placenta or is distributed into milk	The drug may be eliminated by the reticuloendothelial system. Half-life 8-12 days in adults with Crohn's disease or rheumatoid arthritis	Drugs.com-Online resource on Remicade

the future perspective seems to be of high demand in the medical science many protein engineering tools have been developed that allow one to optimize favorable properties of proteins, attenuate undesired attributes and create proteins with entirely novel activities (Carter, 2011). Recombinant protein therapeutic; an important area in therapeutic protein has changed the face of modern medicine in the past decades and continues to provide innovative and effective therapies for numerous human diseases ranging from cancers to infertility (CHO Consortium).

CONCLUSION

It is well accepted that the protein therapeutics market has shown a healthy growth in past few years. Advances in recombinant DNA technology have contributed significantly for this growth. According to global protein therapeutic market analysis by RNCOS Industry Research Solutions in 2011 (Global Protein Therapeutics Market Analysis, 2011), global protein therapeutic market is expected to at a growth rate around 13% during 2012-2014. It is also criticized at the same time that the scientific and analytical technologies for therapeutic proteins have not achieved the level of sophistication as for small molecules. Protein therapeutics will surely rule the medical science in future world, but more in depth research, advancement in technology as well as better understanding of the mechanism of action of protein drug, their pharmacokinetics and pharmacodynamics exploration is required.

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