Proteins therapy for drug discovery

Proteins are most dynamic and diverse macromolecules in our body, thus numerous functionally distinct proteins hold enormous promise for the development of new therapeutics for a variety of human ailments which contain mutated or other abnormal proteins, or those in an abnormally high or low concentration. However, the clinical application of protein therapeutics is still in its infancy since the poor physicochemical stability of proteins in the circulation and their limited membrane permeability interrupt successful delivery to the target sites. This review discusses advantages and limitations of current strategies, as well as the recent developments in protein delivery using nanoparticles. We also highlight nanoparticle-mediated alternative administration routes to injection, including oral, nasal, pulmonary, and transdermal delivery.

Keywords: nanoparticles, protein delivery, protein therapeutics, administration routes, drug delivery systems

1. Introduction

With the strong growth in biopharmaceuticals and advanced drug delivery technologies in recent years, pharmaceutical companies are increasingly turning toward protein therapeutics in the search for drug discovery targets. A study by BCC Research indicated that the global market for bioengineered protein drugs was valued at $151. 9 billion in 2013 and the market is further expected to grow to about $222. 7 billion in 2019 for a compound annual growth rate (CAGR) of 7. 2% from 2014 through 2019 [1]. Compared with the conventional small-molecule drugs that currently make up the majority of the pharmaceutical market, protein drugs offer the advantages of high specificity and less toxicity, whereas the high specificity often requires

structural complexity of the proteins which can make them difficult to formulate, as well as challenging to deliver proteins to target disease sites. Nanotechnology-based approaches, including drug delivery systems using nanostructures such as liposomes, polymer nanoparticles, metallic nanoparticles, stimuli-responsive nanoparticles, and nanofabricated devices, has improved therapeutics in the field of biomedical applications [2, 3]. This review describes current protein delivery technologies including those in the market, recent progress, and unmet needs in the formulations and delivery of proteins. The advances in nanotechnology reviewed here highlight that major hurdles in protein delivery can be met even through the patient-friendly, non-invasive routes.

2. Progress and challenges in protein delivery

To achieve successful protein therapeutics, the intrinsic characteristics of proteins such as structural instability and short half-life should be improved by designing appropriate protein delivery platforms. Inadequate design or formulation of protein drugs can cause degradation, denaturation, and/or aggregation of the protein molecules, and these could potentially cause immunogenic side effects after administration as well as lead to a loss in pharmacological activity. Effective intracellular protein delivery also remains a challenge as hydrophilic and large sizes of proteins are hardly permeated through the cell membrane. In this section, current technologies to deliver proteins, including intracellular delivery strategies, and their limitations will be discussed.

1. *Current protein formulations and modifications*

Biodegradable microparticles (1-1000 ï ¼m) are attractive parental depot formulations for long-term protein drug release (from week to month). They enable sustained release of the proteins by both the diffusion of proteins from the polymer matrix and the degradation/erosion of the polymer [4, 5]. The most widely used material for the encapsulation of proteins is poly(lactic-co-glycolic acid) (PLGA), as they are mechanically strong, biocompatible, biodegradable with favorable degradation rates, non-toxic, and approved for use in humans by the US Food and Drug Administration (FDA) [6]. Encapsulation of proteins into the microparticles can be prepared by several methods such as double emulsion, which is most widely used technique, phase separation (coacervation), ultrasonic atomization, spray-drying, microfluidics, etc. [7]. Once the proteins are encapsulated into microparticles, their release kinetics depend on the microparticle size, molecular mass of polymer, ratio of hydrophilicity/hydrophobicity, polydispersity of microparticle size, and loading amount of proteins. Generally, larger size of microparticles lead to more prolonged protein release, but they can cause potential blockage of the needle required for administration, also the stability and bioactivity of the released proteins in the physiological condition need to be considered for long-term delivery. Degradation and erosion of PLGA can lower the pH inside the microparticles, which can further bring denaturation of the protein as well as aggregate formation. Currently, there are few microparticle drug delivery formulations (e. g. Trelstar depot) on the market and various microparticles have been designed for therapeutic protein delivery such as bone morphogenetic protein-2 [8], insulin [9], recombinant human epidermal growth factor [10], and recombinant human erythropoietin (EPO) [11].

Proteins smaller than 70 kDa are mostly cleared from the systemic circulation by glomerular filtration [12]. Chemical modification of proteins with hydrophilic polymers can reduce this renal clearance by increasing their molecular weight and/or hydrodynamic dynamic radius. The covalent attachment of polyethylene glycol (PEG) chains to proteins (PEGylation), as a typical example, enhances protein stability and pharmacokinetic (PK) properties, and the benefits of PEGylation have the PEGylated therapeutic proteins have reached the market with many examples on various stages of clinical development including Naloxegol (Movantik ^{TM} ; AstraZeneca) which was approved by FDA in 2014 for the treatment of opioid-induced constipation [13, 14]. Hyperglycosylation can also extend biological half-life and improve stability by improving solubility of proteins and reducing immunogenicity. The addition of sugar molecules to a protein is more natural process than PEGylation since it is already a part of endogenous post-translational enzymatic process as well as polysaccharides are readily degraded into native glucose molecules [15]. N-glycosylated EPO (Aranesp) is marketed by Amgen from 2001, and there are more glycosylated protein drugs under preclinical and clinical investigation such as polysialylated forms of EPO, granulocyte-colony stimulation factor (G-CSF), and insulin [16]. Although the chemical modification provides the prolonged circulation half-life of the proteins, this approach can result in unfavorable conformational changes, a loss of biological activity and binding affinity to their target due to steric hindrance, and heterogeneity [17]. This reduction in physicochemical properties leads to the systemic exposure of proteins to get enough pharmacological potency, but toxicities related to peak exposure can limit their clinical use. Various efforts aiming for the maintenance of protein

activity are being made by designing site-specific modification. For example, chemical ligation of synthetic peptides including levulinyllysine to EPO indicated superior hematopoietic activity compared to native protein [18]. More recent advances in chemoselective targeting show that the incorporation of canonical and noncanonical amino acids can enhance the selectivity, while improving PEG architecture [19].

In addition to chemical modification, genetic constructs and fusion technologies have been intensively studied to elevate protein half-life and delivery efficacy. Fc-based fusion proteins that are composed of an immunoglobin Fc domain and genetically linked therapeutic protein to this domain are promising approaches as Fc-fusion can endow a protein with unique effector functions mediated by Fc receptor binding and complement fixation [20]. The neonatal Fc receptor (FcRn) mediated recycling and transcytosis process results in half-life extension (e. g. IgG: up to 21 days) and also the increased molecular weight of fusion proteins through the size of the Fc-domain (~50 kDa) reduces renal clearance [21]. A number of therapeutic proteins based on fusion with the IgG Fc domain are on the market for clinical use since Fc-fused tumor necrosis factor (TNF) receptor-2 (Enbrel; Amgen/Pfizer) was approved for the treatment of rheumatoid arthritis and plaque psoriasis in 1998, and several candidates are currently under clinical trials [22]. Recent Fc-fusion platforms focus on the ways to retain biological activity and binding affinity which can be commonly decreased after fusion process [23, 24]. Jung et al. included a ‘ chaperone’ protein in Toll-like receptor 4 Fc-fusion to stabilize the desired partner [25]. The development of heterodimeric Fc platforms based on strand-exchange

engineered domain CH3 heterodimers consisted of alternating segments of human IgA and IgG CH3 shows multiple specificities within homodimeric Fc-fusion platform [26]. To utilize alternative backbones, such as IgA, IgE, and IgM, may also serve benefits to the activity of the fused partner [27-29]. However, concerns are ongoing about the immunogenicity of Fc-fusion proteins because interactions between the Fc domain and its receptors have multivariable immunological consequences, which can raise concerns in the treatment for chronic disease [30]. Other attempts to target FcRn including albumin fusion which has direct interaction with FcRn and genetic engineering of Fc domains have also been reported. A glucagon-like peptide-1 (GLP-1) albumin fusion achieved ~ 5 day half-life and received FDA-approval (Albiglutide; GSK) for the treatment of type-2 diabetes [31]. A recombinant polypeptide fusion construct which consists of an unstructured polypeptide and protein drug is another example of generic fusion technology capable of extending plasma half-life. Schellenberger et al. developed an exenatide-XTEN fusion and demonstrated ~58 times increased half-life and a low rate of immunogenicity in animals, even in the presence of the adjuvant [32]. Still, issues remain in safety of fusion approaches, in particular in the case of fusions with native human proteins because of the cross-reactivity with endogenous homologues which can affect on a long-term safety and clearance of subsequent doses [33].