Anatomical, Physiological, and Experimental Factors Affecting the Bioavailability of sc-Administered Large Biotherapeutics

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ABSTRACT: Subcutaneous route of administration is highly desirable for protein therapeutics. It improves patient compliance and quality of life (McDonald TA, Zepeda ML, Tomlinson MJ, Bee WH, Ivens IA. 2010. Curr Opin Mol Ther 12(4):461–470; Dychter SS, Gold DA, Haller MF. 2012. J Infus Nurs 35(3):154–160), while reducing healthcare cost (Dychter SS, Gold DA, Haller MF. 2012. J Infus Nurs 35(3):154–160). Recent evidence also suggests that sc administration of protein therapeutics can increase tolerability to some treatments such as intravenous immunoglobulin therapy by administering it subcutaneously (subcutaneous immunoglobulin therapy SCIG), which will reduce fluctuation in plasma drug concentration (Kobrynski L. 2012. Biologics 6:277–287). Furthermore, sc administration may reduce the risk of systemic infections associated with i.v. infusion (McDonald TA, Zepeda ML, Tomlinson MJ, Bee WH, Ivens IA. 2010. Curr Opin Mol Ther 12(4):461–470; Dychter SS, Gold DA, Haller MF. 2012. J Infus Nurs 35(3):154–160). This route, however, has its challenges, especially for large multidomain proteins. Poor bioavailability and poor scalability from preclinical models are often cited. This commentary will discuss barriers to sc absorption as well as physiological and experimental factors that could affect pharmacokinetics of subcutaneously administered large protein therapeutics in preclinical models. A mechanistic pharmacokinetic model is proposed as a potential tool to address the issue of scalability of sc pharmacokinetic from preclinical models to humans. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:301–306, 2015

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INTRODUCTION

Protein therapeutics are classified based on their pharmacological function into (1) proteins with enzymatic/regulatory function or (2) proteins with targeting function (monoclonal antibodies).[1] The first class contains proteins ranging in size from small peptide hormones such as insulin and erythropoietin to the large multidomain proteins such as FVIII and acid alpha-glucosidase (GAA). These therapeutics are designed to: (1) replace lacking or aberrantly formed endogenous counterparts to ameliorate disease conditions such as the use of insulin in diabetes; (2) augment existing pathways such as the use of human follicle-stimulating hormone for infertility; and (3) provide a novel function such as hyaluronidase.[2,3] The second class contains monoclonal antibodies (mAbs) and their derivatives. This class of protein therapeutics is characterized by unique pharmacokinetics because of their high-target-binding affinity and the presence of the Fc fragment (in the case of mAb), which imparts the prolonged half-life of this class of biologics.

The wide range in the size and properties of protein therapeutics makes it difficult to treat them as a single class of therapeutics, especially when discussing sc absorption. Furthermore, the classification of protein therapeutics based on pharmacological function may be irrelevant when discussing absorption from the subcutaneous space. This necessitates a different categorization system based on the size rather than the function of these therapeutics. The following sections discuss the physical barriers to sc absorption of protein therapeutics, which should help in classifying protein therapeutics, based on size, into (1) small proteins of less than 10 nm in diameter, (2) large proteins of greater than 10 nm in diameter, and (3) mAbs. Next, we discuss presystemic degradation as a contributing factor to incomplete bioavailability before presenting possible experimental artifacts in preclinical models that can further contribute to poor scalability to humans.

BARRIER TO sc ABSORPTION OF PROTEIN THERAPEUTICS

Physical Barriers

After a drug is deposited in the sc space, it must traverse the extracellular matrix to reach an entry point into systemic circulation. Entry can be directly into the blood stream or by transiting through the lymphatics.[4]

Direct Uptake into Blood

Uptake into blood requires entry at the postcapillary bed or by traversing the basal membrane of blood vesicles, both of which are size limiting. The postcapillary bed is involved in blood/tissue fluid exchange, it is also the primary site of leukocytes and plasma protein leakage.[5] These capillaries preferentially reabsorb particles up to 10 nm.[6] Alternatively, the drug enters systemic circulation by crossing the basal membrane of blood vessels via the paracellular or transcellular pathway. The former is limited by the size of the fenestrations in the basal membrane reported to be 6–12 nm for most nonsinusoidal blood capillaries.[7]

The transcellular pathway may not be a major player in protein uptake. Indeed, large proteins have been shown to have poor transcellular trafficking.[8] Those therapeutic proteins are generally hydrophilic, which prevents them from traversing the

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cell membrane. Protein entering through pinocytosis or phagocytosis will likely be degraded leading to the loss of protein. One exception is monoclonal antibodies. Transcellular transport of mAbs has been recognized since the early 1970s.[8] This is mediated by FcRn receptors on the surface of endothelia cells. FcRn not only facilitates the bidirectional transport of mAbs,[9,10] but it also protects the antibody during fluid phase pinocytosis by binding the antibody and sorting it away from the lysosomal pathways.[11–13] FcRn-mediated transport explains the high bioavailability and the saturable nature[14] of mAb uptake from sc.

Physicochemical properties of antibodies that can potentially affect transcellular trafficking of mAbs such as isotype, FcRn-binding affinity, charge, hydrophobicity, and solubility have been investigated by a number of researchers in the field with conflicting results. For example, Khawli et al.[15] showed no effect of charge variants of IgG1 on their pharmacokinetics after sc administration in rats. This, however, is in contrast to other reports showing that alteration to the isoelectric point of mAbs altered the bioavailability after sc administration in mice.[16] Interestingly, both groups reported no change in FcRn-binding affinity as a function of changes in pI of the protein.[15,16]

The role of pI and protein surface charge in sc uptake could be explained by charge–charge interaction during fluid phase pinocytosis. It has been shown that IgGs with higher pI have higher cellular uptake.[17,18] This suggests that a positively charged IgG interacts more favorably with the negatively charged cell surface allowing for more uptake of mAb during fluid phase pinocytosis[16–18]; the IgG will then bind to FcRn, which will protect it from degradation. Negatively charged IgG, on the contrary, will have lower uptake because of the repulsion between the negative protein and the negative cell surface.[16] However, in the context of the sc space, the repulsive forces between the negative protein and the negative extracellular matrix could enhance convective movement of the protein through the extracellular matrix and improve lymphatic trafficking, as we will discuss below.

Uptake by the Lymphatics

Uptake by lymphatics is less restrictive. The initial lymphatics, where interstitial fluid enters the lymphatic system and becomes lymph fluid, do not have a continuous basal membrane.[6,19] Rather, the endothelial cells of the initial lymphatic overlap while being anchored by collagen VII to the extracellular matrix.[6,19] The lack of the basal membrane allows for large proteins as well as small cells, bacteria, and viruses to enter the lymphatics.[19] Anchorage to the extracellular matrix, on the other hand, allows for the transmission of mechanical forces from the extracellular matrix to the lymphatic lumen.[19] This can allow the initial lymphatics to open up in response to mechanical movement; this can explain the improved lymph flow in response to massaging or movement.

Despite the lax size limitation of lymphatic uptake, there are still a number of other impediments to absorption of biologics via this route. After injection, the protein must navigate the extracellular matrix to reach a point of entry into the lymphatics. The density of the initial lymphatics at the injection site[4] will affect the proficiency of lymphatic uptake of protein from the injection site. This process can also be affected by the size and charge of the proteins.[6] Larger proteins are selectively taken up by the lymphatics; however, the larger the protein, the slower the uptake[6] because of the increased resistance to convective and/or diffusive movement. Also, electrostatic interaction with glycosaminoglycanes, the negatively charged component of the glycocalyx matrix,[5,6] can hide or promote the movement of the protein through the extracellular matrix.[6,20] Indeed, positively charged proteins have been reported to reach the lymphatics at a delayed time as compared with negatively charged protein of comparable size.[21]

It is important to note that the above-mentioned uptake pathways are not mutually exclusive, and protein absorption can occur via one or more of the pathways discussed above. For example; small protein therapeutics can utilize the postcapillary bed as well as the fenestrations in the basal membrane of blood vessels; this explains their good bioavailability. mAbs can utilize FcRn receptors on the surface of endothelial cells as well as lymphatic uptake. Large protein therapeutics, however, must utilize lymphatic uptake.

Strategies that can overcome one or more of the abovementioned physical barriers could enhance bioavailability of protein therapeutics. For example, the use of hyaluronidase to "loosen" the extracellular matrix enhances the diffusion of the coadministered biologics.[3] Another example is the use of albumin to manipulate the oncotic pressure, and by extension the interstitial fluid volume in sc space, to enhance sc bioavailability.[22,23] Other strategies to manipulate the environment in the sc space to improve overall bioavailability of protein therapeutics such as viscosity, osmolarity, and volume of injection have been recognized by a number of workers in the field and are discussed in a number of reviews.[4,24]

In our own work, we found a relationship between increased buffer tonicity and improved bioavailability of subcutaneously administered rituximab in a mouse model.[25] Furthermore, our data suggest that the effect of buffer hypertonicity on rituximab bioavailability is excipient specific. We found that mannitol, a neutral excipient, performed better than the negatively charged O-phospho-L-serine at equal tonicities. The enhanced bioavailability was associated with enhanced lymph node uptake of rituximab.[25] We propose that hypertonic buffers perturb the isotonicity of the interstitial space altering the formation and reabsorption of interstitial fluid at the postcapillary beds. The draining of excess interstitial fluid by the lymphatics enhances bulk movement of fluid through the sc space carrying with it the protein from the injection site through to the lymphatic.[25]

Presystemic Degradation

Another complicating factor for sc absorption of protein therapeutics is presystemic elimination.[4,14] This could be due to degradation at the injection site by proteolytic enzymes. The presence of such enzymes is supported by reports showing this proteolytic activity in the sc space to be saturable by administering high doses of the drug as well as inhibited by coadministering protease inhibitors.[14,26,27] This proteolytic activity has been proposed as a reason for incomplete bioavailability of biologics. Another form of presystemic elimination is the uptake and processing of protein therapeutics by professional antigen presenting cells in the skin. Upon sc administration, dermis and epidermis resident dendritic cells migrate to the injection site to sample the injected protein.[28] This leads to maturation of these