

Special Section on DMPK of Therapeutic Proteins—Minireview

Subcutaneous Absorption of Biotherapeutics: Knowns and Unknowns

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ABSTRACT

Subcutaneous administration of biotherapeutics offers several potential advantages compared with intravenous administration. Many biotherapeutics, both marketed or in development, are administered via the subcutaneous route. This minireview provides an overview of the presystemic absorption processes following subcutaneous administration, the resulting pharmacokinetics after subcutaneous administration, and provides recent case examples of the development of subcutaneous administered drugs with a focus on monoclonal antibodies. Subcutaneous absorption of biotherapeutics is relatively slow and mostly incomplete. Knowledge of the subcutaneous tissue is important to understand the absorption kinetics after

subcutaneous administration. Transport in the subcutis to the absorbing blood or lymph capillaries appears to be a major contributor to the slow subcutaneous absorption. Larger proteins (>20 kDa) are mostly absorbed via the lymphatic system, although potential species differences are not fully understood yet. Also, the presystemic catabolism leading to incomplete bioavailability is little understood, both the involved enzymes and its translation across species. For IgGs, binding to the neonatal Fc receptor is important to obtain a high bioavailability. Overall, several aspects of subcutaneous absorption are still poorly understood, which hampers, e.g., translation across species. Further research in this area is warranted.

Introduction

Numerous biotherapeutics are marketed or currently being developed for many diseases and disorders, particularly as anticancer or anti-inflammatory agents. Until now, approaches for oral administration of biotherapeutics have failed, so that biotherapeutics have to be administered via the parenteral route. Subcutaneous administration offers several potential advantages compared with the intravenous route of administration. Whereas intravenous infusions usually have to be administered in a hospital or in a doctor's office, subcutaneous administration may be performed by a health care professional at the patient's home or even by patient self-administration (Schweighofer and Wendtner, 2010). In addition, subcutaneous administration helps to treat patients with poor venous access or to spare patients' venous capital (Launay-Vacher, 2013). Subcutaneous administration is of particular benefit for long-term or chronic drug treatments. In addition, subcutaneous administration may be better tolerated as compared with intravenous administration, as the slow absorption may abrogate side effects related to high serum concentrations. This was demonstrated for alemtuzumab (Hale et al., 2004). An overview of marketed subcutaneous administered monoclonal antibodies (mAbs) and IgG fc-fusion proteins is provided in Table 1. Subcutaneous administration may offer additional advantages for biotherapeutics with short residence time in the body. The

slow absorption after subcutaneous administration may lead to an absorption-rate-limited pharmacokinetics and, thus, to a prolonged systemic exposure (Mager and Jusko, 2002). Drawbacks of subcutaneous administration include the incomplete bioavailability after subcutaneous administration (Richter et al., 2012). The relatively slow subcutaneous absorption is also of note, particularly when rapid onset of action is required.

Following intravenous administration, a biotherapeutic is directly injected into the systemic circulation. Following subcutaneous administration, however, the biotherapeutic is injected into the extracellular space of the subcutaneous tissue; from there it has to be transported to blood or lymph capillaries for absorption, prior to reaching systemic circulation. These processes are influenced both by properties of the biotherapeutic and by host factors (Richter et al., 2012). These presystemic events have to be considered in understanding the subcutaneous administration of biotherapeutics.

The subcutaneous absorption of biotherapeutics has been summarized in a few recent reviews (McDonald et al., 2010; Richter et al., 2012). In the present review, we provide an overview of the knowns of presystemic absorption processes following subcutaneous administration with a focus on mAbs, the resulting pharmacokinetics after subcutaneous administration, and provide recent case examples on the development of subcutaneous administered drugs. In addition, we summarize some of the unknowns in subcutaneous absorption of biotherapeutics.

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ABBREVIATIONS: ECM, extracellular matrix; EPO, erythropoietin; FcRn, neonatal Fc receptor; GAG, glycosaminoglycan; hGH, human growth hormone; mAb, monoclonal antibody; PEG-EPO, pegylated erythropoietin; PK, pharmacokinetic; rHuPH20, human recombinant hyaluronidase; T_{max} , time to maximum serum concentration; TMDD, target-mediated drug disposition; VEGF-C156S, vascular endothelial growth factor Cys156Ser.

TABLE 1

Overview of marketed IgGs and IgG Fc-fusion proteins administered via the subcutaneous route

Data on time to maximum serum concentration and bioavailability collected from the European Medicines Agency (EMA) Summary of Product Characteristics (http://www.ema.europa.eu/ema/index.jsp?url=pages/home/Home_Page.jsp&mid=), EMA assessment reports, or the Food and Drug Administration homepage (<http://www.accessdata.fda.gov/scripts/cder/drugsatfda>).

INN Name	Trade Name	Antibody Type	Target	T _{max} in Humans	Bioavailability in Humans and Animals	
					days	%
Adalimumab	Humira	IgG1	TNF	5.5	Humans: 64	Cynomolgus monkey: 96
Canakinumab	Ilaris	IgG1	IL-1 β	7	Humans: 70	Marmoset: 60
Denosumab	Prolia	IgG2	RANK-L	10	Humans: 78	Mice: 86
Golimumab	Simponi	IgG1	TNF	2–6	Humans: 53	Cynomolgus monkey: 28–100 ^a
Omalizumab	Xolair	IgG1	IgE	7–8	Humans: 62	Cynomolgus monkey: 77
Rituximab	Mabthera	IgG1	CD20	3	Humans: 62	Mice: 90
Tocilizumab	Actemra / RoActemra	IgG1	IL-6R	—	Cynomolgus monkey: 64–104	Humans: 71
Trastuzumab	Herceptin	IgG1	HER2	3	Humans: 71	Mice: 63
Ustekinumab	Stelara	IgG1	IL-12/23p40	8.5	Minipig: 71	Humans: 80
Abatacept	Orencia	Fc-fusion protein	CD80/86	3–5	Humans: 80	Minipig: 84
Etanercept	Enbrel	Fc-fusion protein	TNF	3	Cynomolgus monkey: 72	Humans: 82
Rilonacept	Arcalyst	Fc-fusion protein	IL-1 α , IL-1 β , IL-1ra	—	Mice: 83	Minipigs: 82
Romiplostim	Nplate	Fc-fusion protein	Thrombopoietin receptor	0.6	Cynomolgus monkey: ca. 100	Humans: 57 (24–95)
					Humans: 79	Cynomolgus monkey: 97
					Mice: 78–110	Humans: 79
					Rat: 41–63	Mice: 78–110
					Humans: 76	Rat: 41–63
					Mice: 58	Humans: 76
					Cynomolgus monkey: 73	Mice: 58
					Humans: 43	Cynomolgus monkey: 73
					Mice: 78	Humans: 43
					Rats: 54	Mice: 78
					Cynomolgus monkey: 70	Rats: 54
					Humans: –	Cynomolgus monkey: 70
					Rats: 21–28	Humans: –
					Cynomolgus monkey: 19	Rats: 21–28
					Rhesus monkey: 45–74	Cynomolgus monkey: 19
						Rhesus monkey: 45–74

CD, Cluster of Differentiation; HER2, human epidermal growth factor receptor 2; IL, interleukin; INN, international nonproprietary name; RANK-L, receptor activator of nuclear factor- κ B ligand; TNF, tumor necrosis factor.

^aBioavailability increases with increasing dose.

Presystemic Transport Processes following Subcutaneous Administration

Subcutaneous administration delivers the dosed biotherapeutic into the subcutaneous tissue (hypodermis), where the drug material resides and is transported until being absorbed into blood or lymphatic capillaries. Thus, the hypodermis is of key interest in understanding the absorption behavior of biotherapeutics after subcutaneous administration. The structure of the hypodermis differs across species. The hypodermis in humans was discussed in some detail in a recent review (Richter et al., 2012). In brief, the human hypodermis consists of adipose tissue, i.e., fat lobules that are separated by septa of loose (areolar) connective tissue. The main cellular components of the hypodermis are adipose cells and, to a minor extent, fibroblasts and macrophages. Fibroblasts produce components of the extracellular matrix (ECM) such as collagen and glycosaminoglycans (GAGs).

The connective tissue septa represent the majority of the interstitial space in the hypodermis. In the septa, a fibrous collagen network links the dermis to the deep fascia covering the skeletal muscle underneath and, thus, maintains the mechanical structure of skin. The composition of the interstitium, i.e., both interstitial matrix and interstitial fluid,

across tissues has been extensively summarized in a recent review and, thus, will not be discussed in detail (Wiig and Swartz, 2012). In brief, the interstitial matrix contains collagen, GAGs, and proteoglycans. The amount of elastin, another component of the interstitial matrix, is low and of little relevance in the context of subcutaneous absorption. GAGs are highly negatively charged polysaccharides, which consist of repeating disaccharide units of N-substituted hexosamine and uronic acid. Hyaluronic acid, also referred to as hyaluronan, is the most common GAG in the hypodermis and consists of disaccharide units of N-acetylglucosamine and glucuronic acid. The strongly negatively charged GAGs control the interstitial fluid content and hydraulic conductivity of the interstitium (Aukland and Reed, 1993; Wiig and Swartz, 2012).

After administration, the biotherapeutic must be transported through the interstitium to reach blood or lymph capillaries. Transport through the interstitium may involve diffusion and convection (Swartz, 2001). The contribution of diffusion and convection to transport depends on the molecular weight/size of the solute and the GAG content in the interstitium (Swabb et al., 1974). Higher GAG content and increasing molecular weight favor transport by convection. Thus, transport of

large proteins such as albumin or IgGs in subcutaneous tissue is dominated by convection (Swabb et al., 1974; Reddy et al., 2006). Convective transport is driven by fluid flow from capillaries to the lymphatic system. The driving forces for the fluid flows are differences in hydrostatic and osmotic pressure between blood circulation, interstitium, and the lymphatic system, which are commonly referred to as Starling forces (Wiig and Swartz, 2012). Thus, fluid efflux from arterial capillaries (arterioles) into interstitium is governed by hydrostatic pressure differences between arteriole and interstitium as well as by differences in colloid osmotic pressure between plasma and interstitium. Some of the fluid is reabsorbed into venous capillaries (venules). The reuptake into blood circulation is driven by the lower pressure in venules as compared with the interstitium. Fluid that was not reabsorbed into venules is convected through the interstitium and taken up by lymphatic capillaries. The balance between venous and lymphatic fluid uptake has been debated. It has been suggested that the majority of the arterial exudate is reabsorbed into postcapillary venules (Swartz, 2001), whereas other views suggest that the lymphatic uptake exceeds reuptake into venous circulation (Waterhouse et al., 2010).

Convective transport in the interstitium is influenced by the size and charge of the molecule. Studies on interstitial transport in the dermis of mouse tails showed that convection of dextrans in a molecular mass range from 3 to 71 kDa is dominated by size exclusion (i.e., similar to transport in a size exclusion chromatography column, solutes with a higher Stokes-Einstein radius are transported faster than smaller solutes) (Reddy et al., 2006). The slower interstitial transport of small solutes does not necessarily lead to a slower absorption into systemic circulation, as small solutes can be absorbed into blood circulation rather than into the lymphatic system (see below). For very large molecules such as a 2000-kDa dextran, steric hindrance from the interstitial gel matrix slows convection. The negative net charge of the interstitial matrix leads to a more rapid transport of negatively charged molecules compared with positively charged molecules (Reddy et al., 2006). In line with these findings, Mach and coworkers (2011) reported that positively charged monoclonal antibodies bind to rat subcutaneous tissue *in vitro* in a way that is consistent with electrostatic interactions. It is of note that the binding could be saturated by highly concentrated protein solutions (30–50 mg/ml).

During transport through the interstitial space, a drug molecule can access only a part of the interstitial space volume. Matrix molecules such as collagen and hyaluronic acid occupy a certain volume fraction of the interstitial space, which cannot be accessed by other compounds. This so-called exclusion volume increases with both molecular weight and negative charge (Reddy et al., 2006). The exclusion volume for albumin was reported to be ca. 30% of the total interstitial volume for rabbit subcutaneous tissue (Negrini et al., 2003).

Subcutaneous absorption is also influenced by host factors. Movement, heat, massage, and other factors can enhance subcutaneous absorption (Richter et al., 2012). A summary of these host factors is beyond the scope of this minireview.

Absorption via the Lymphatic System

Larger proteins are predominantly absorbed via the lymphatic system (McLennan et al., 2005). Absorption into the lymph system usually occurs at the initial lymphatics (i.e., lymphatic capillaries and precollectors). A plexus of lymph capillaries is located in the dermis at a depth of ca. 200 μm (Ryan, 1989; Lubach et al., 1991). These lymph capillaries drain into lymph precollectors, which form a second plexus at the dermis-subcutis junction. From there, lymph drains through lymph collectors in the connective tissue septae of the subcutis to the next lymph node. The contribution of the individual parts of lymphatics in the dermis and subcutis to the lymphatic absorption of subcutaneous

administered biotherapeutics remains to be clarified. Thus, it is unknown whether the plexus in the dermis contributes to the absorption. The lymphatic capillaries are blind-ended and composed of a single layer of overlapping endothelial cells, and lack tight cell-cell junctions as well as a continuous basement membrane. Usually, lymph capillaries are collapsed. Lymphatic endothelial cells are linked via anchoring filaments to collagen fibers of the extracellular matrix (Swartz, 2001). Increase in interstitial pressure stretches the fibers and leads to an opening of the lymphatic lumen (Skobe and Detmar, 2000). The loose connection between the endothelial cells allows easy entry of fluids and macromolecules into the lymphatic capillary. Thus, in contrast to blood capillaries, they allow an easy entry of large-molecular-weight solutes such as proteins, which favors the lymphatic absorption of large biotherapeutics. There is little if any exclusion of any interstitial protein during transport from interstitial space into the lymph capillary, so that the primary lymph has almost the same composition as interstitial fluid (Swartz, 2001). Primary lymph flows from the capillaries via collecting ducts to a regional lymph node. In the lymph node, fluid exchange occurs to equilibrate Starling forces (hydrostatic and colloid-osmotic pressure) between lymph and blood capillaries (Aukland and Reed, 1993). About half of the water from lymph may be reabsorbed in blood (Waterhouse et al., 2010), so that the concentrations of solutes in postnodal lymph may exceed those in interstitial fluid and primary lymph. Postnodal lymph from the majority of the body is collected in the thoracic duct, which empties into the left subclavian vein. Lymph from the upper-right quadrant of the body is not returned via the thoracic duct to blood circulation, but rather directly into the right subclavian vein. Species differences in the lymphatic system, however, need to be considered. In rats, for instance, the lymphatic drainage routes differ from the aforementioned one in humans, in that lymph from major parts of the body is returned via the subclavian duct, bypassing the thoracic duct (Tilney, 1971).

The role of the lymphatic system in subcutaneous absorption of therapeutic proteins has been extensively studied in a sheep model (Supersaxo et al., 1990; McLennan et al., 2002). Following subcutaneous administration, the cumulative recovery in peripheral lymph (popliteal lymph node) draining the administration site at a hind leg increased with molecular weight (Fig. 1). Starting at molecular mass around 20 kDa, the majority of dose is absorbed via the lymphatic route. Almost complete absorption via the peripheral lymph was observed for proteins with molecular mass of ca. 40 kDa and above. Lymphatic absorption has been much less studied in other species, so

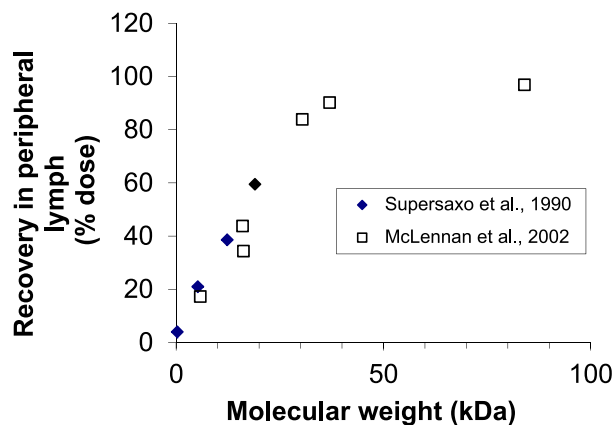


Fig. 1. Fraction of dose (mean, $n = 3-4$) recovered in peripheral lymph following subcutaneous administration to sheep for a series of small molecular compounds and proteins with molecular mass ranging from 0.25 to 84 kDa (data from Supersaxo et al., 1990; McLennan et al., 2002).

that it remains to be clarified whether the relationship between molecular weight and lymph uptake in sheep is also valid for other species. Only limited data are available on lymphatic absorption of macromolecules in rat, rabbit, or dog. Wang and coworkers (2012) reported relevant lymphatic recovery of ^{125}I -labeled pegylated erythropoietin (PEG30-EPO and PEG40-EPO) in lymph from thoracic duct-cannulated rats and dogs. In rats, lymphatic recovery of protein-associated radioactivity over 7 days accounted for approximately 60–70% of bioavailable material in noncannulated controls. In a thoracic duct-cannulated dog, lymphatic recovery of protein-associated radioactivity accounted for 20% of the administered dose over a period of 7 days. Zou and coworkers (2013) reported relevant lymphatic recoveries in thoracic duct-cannulated dogs and rats (73 and 27% of dose, respectively) after subcutaneous administration of a 48-kDa pegylated peptide. Considering the observed presystemic catabolism in the rat, the lymphatic recovery of 27% of dose reflects approximately half of the bioavailable material. Other data on lymphatic absorption of proteins in rats, however, have been controversial. Kagan and coworkers (2007) reported a low lymphatic recovery from the thoracic duct (less than 3% of dose) after subcutaneous administration of bovine insulin, bovine serum albumin, and EPO. Kojima and coworkers (1988) had similar findings for tumor necrosis factor in rats. It remains to be clarified whether the lymphatic absorption differences in rats reflect a species difference from, e.g., sheep or interexperimental variability due to incomplete lymph sampling owing to the different lymphatic system in the rat (see earlier discussion).

Only a few studies on lymphatic absorption of mAbs have been reported. Following subcutaneous administration of trastuzumab to thoracic duct-cannulated rats, 27% of dose was recovered within 30 hours (Dahlberg et al., 2014). By this time, absorption was not complete, so that the lymphatic recovery underestimates lymphatic absorption. A compartmental pharmacokinetic model suggests that 53% of the trastuzumab dose is absorbed in a first-order process into peripheral lymph. Relevant lymphatic absorption was also reported for the IgG fusion protein lenercept in thoracic duct-cannulated rabbits (Richter et al., 2014). Cumulative recovery in lymph over 48 hours following subcutaneous administration ranged from 10 to 17% of dose, which is equivalent to approximately 25–40% of subcutaneous bioavailable material in noncannulated rabbits.

What Determines the Slow Absorption after Subcutaneous Dosing?

Subcutaneous absorption of protein, particularly mAbs, is slow, as indicated by the time to maximum serum concentration (T_{max}) usually ranging from around 3 to up to 8 days in humans (Table 1). T_{max} of several days values for mAbs are also observed in larger animals such as cynomolgus monkeys or minipigs (Fig. 2; Zheng et al., 2012). Reasons for slow absorption may include slow drug transport through the ECM prior to reaching capillaries, or a slow lymph flow. Zhao and coworkers (2013) developed a hybrid pharmacokinetic (PK) model to describe subcutaneous absorption and subsequent disposition. Sensitivity analysis demonstrated that only the parameter “lymph flow” had an impact on T_{max} . In the PK model, the parameter lymph flow linked the “interstitial space” compartment of the injection site with compartment “lymphatic system.” Transport within the interstitial space to the absorbing capillary was not included separately in the structural model, and, thus, reflected in the parameter lymph flow. Accordingly, transport in interstitial space and lymph could not be distinguished in the model. It is not uncommon in the literature to summarize both interstitial transport and transport through the lymphatics as “lymphatic uptake” (Swartz, 2001). Interstitial transport, however, is probably the rate-limiting step during subcutaneous absorption via the lymphatics,

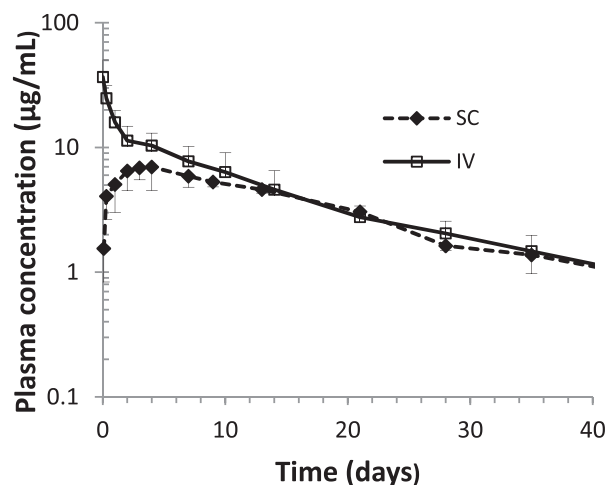


Fig. 2. Dose-normalized plasma concentration-time curves of a mAb following subcutaneous (SC) and intravenous (IV) administration to cynomolgus monkeys (mean \pm S.D., $n = 3$): slow absorption after SC dosing with maximum concentrations reached after 3–4 days.

as the resistance to fluid and solute transport is higher in the ECM as compared with the lymphatic system (Swartz, 2001). Due to the ECM composition, there is resistance to transport through the interstitium by multiple mechanisms (e.g., resistance to fluid flow due to the high viscosity in the interstitium due to tight association of water to hyaluronic acid) (Bookbinder et al., 2006).

Experimental data demonstrate a long residence time at the subcutaneous administration site in comparison with a relatively high lymph flow velocity, which supports interstitial transport as the rate-limiting step in subcutaneous absorption. Wu and coworkers (2012) studied the removal of fluorescence-labeled proteins with molecular mass ranging from 23 to 149 kDa [vascular endothelial growth factor Cys156Ser (VEGF-C156S; 23 kDa), ovalbumin (44.3 kDa), bovine serum albumin (66 kDa), and bevacizumab (149 kDa)] from the subcutaneous administration site in the mouse footpad. Half-lives of drug removal from the subcutaneous administration site were correlated with molecular weight and were as high as 6.81 hours for bevacizumab (0.31, 1.57, 2.85, and 6.81 h for VEGF-C156S, ovalbumin, BSA and bevacizumab, respectively) (Fig. 3), which supports the hypothesis of removal from the subcutaneous administration site as a rate-limiting step. In another study, relevant administration site retention was also demonstrated after dorsal subcutaneous administration of a fluorescence-labeled immunoglobulin to mice (Filipe et al., 2014). Long residence time of subcutaneous administered proteins was also observed in humans. Following subcutaneous administration of [^{131}I]albumin, Hollander and coworkers (1961) observed an initial loss of radioactivity (10–28% of dose) from the subcutaneous dosing site during the first 4–8 hours after administration in 10 of 15 subjects. After the initial loss, radioactivity disappeared from the injection site with a half-life of 33.4 hours (range 18–48 hours). The relatively long residence times at the subcutaneous injection sites need to be compared with residence times in the lymphatic system. A study in the tail microlymphatics of anesthetized mice indicated a median flow velocity of 4.7 $\mu\text{m/s}$ (equivalent to about 0.3 mm/min or 1.7 cm/h) (Berk et al., 1996). Considering the distances traveled in the lymphatics of a mouse, it is unlikely that lymphatic transport is rate limiting for subcutaneous absorption. Experimental findings in humans are in line with these results in mice. Lymph flow in humans was studied following intradermal administration of ^{99}Tc -labeled IgG into the hand (Modi et al., 2007). Transport through the arm lymphatics to the axillary lymph node was imaged using a gamma camera. The lymphatic low velocity from hand to axilla averaged

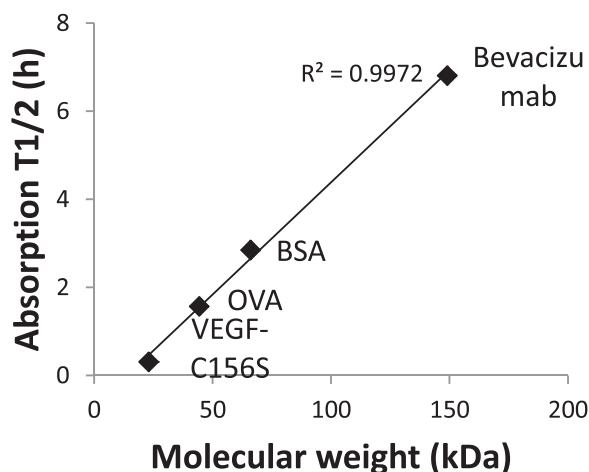


Fig. 3. Half-life of removal from subcutaneous administration site after subcutaneous administration of fluorescence-labeled VEGF-C156S, ovalbumin (OVA), bovine serum albumin (BSA), and bevacizumab in mice (data from Wu et al., 2012).

8.9 ± 5.8 cm/min, and the time interval for lymphatic transport from hand to axilla ranged from 3 to 21 minutes in seven subjects (average 9.6 ± 7.2 minutes). Assuming similar flow rates also in the postnodal lymphatics, the residence time in the lymphatics can be estimated to be in the order of a few hours. With this short estimated lymphatic residence time compared with an absorption duration of days for mAbs (Table 1), transport in the lymphatics is unlikely to be the rate-limiting step for the slow absorption after subcutaneous administration.

Incomplete Bioavailability and Underlying Catabolic Processes

Following subcutaneous administration, the bioavailability of proteins, including IgGs, is usually incomplete (McDonald et al., 2010; Richter et al., 2012). Subcutaneous bioavailabilities in humans are, for instance, 50, 80, and $>89\%$ for interferon β -1b, peginterferon α -2a, and interferon γ -1b, respectively (data from EMA assessments and the FDA [<http://www.accessdata.fda.gov/scripts/cder/drugsatfda/>]). For marketed IgG, the subcutaneous bioavailabilities are mostly around 60–80% (Table 1). The incomplete bioavailability may be caused by first-pass catabolism at the subcutaneous administration site or in the draining lymphatics. Processes of protein first-pass catabolism after subcutaneous administration are still poorly understood.

Local catabolism at the injection site has been demonstrated, for instance, for insulin. Following subcutaneous administration to anesthetized pigs, ^3H -labeled insulin was removed from the injection site with a half-life of 59 minutes (Berger et al., 1979). At all sampling times throughout the experiment (up to 160 minutes postdose), about 20% of radioactivity recovered from the subcutaneous injection site was degradation products, indicating a local catabolism of insulin in subcutaneous tissue. In rat subcutaneous tissue, insulin-degrading activity was mainly found in the 160,000g supernatant fraction of subcutaneous tissue (Komada et al., 1985). Cathepsin B and collagenase-like peptidase were detectable as proteolytic enzymes in rat subcutaneous tissue. Inhibition of proteases in the subcutaneous tissue reduces insulin degradation (Takeyama et al., 1991). Pretreatment of the subcutaneous injection site with ointments containing the protease inhibitors gabexate mesilate or nafamostat mesilate results in a trend toward higher insulin levels in circulation both in rats and healthy volunteers. Nafamostat pretreatment was also reported to successfully overcome the subcutaneous insulin resistance in a diabetes patient. Both insulin absorption and the hypoglycemic effect were markedly increased after nafamostat pretreatment (Kawashima et al., 2008).

For human growth hormone (hGH; molecular mass 22 kDa), loss in the lymphatics was demonstrated as the main reason for incomplete subcutaneous bioavailability (Charman et al., 2000). In noncannulated sheep, the subcutaneous bioavailability was estimated at $58.4 \pm 9.1\%$ (mean \pm S.E.M.). Lymph cannulation was performed either in the efferent lymph vessel from the popliteal node or in the thoracic duct (peripheral and central lymph collection, respectively). Subcutaneous bioavailability in lymph-cannulated sheep was reduced to ca. 30–40%, indicating a relevant contribution of lymphatic absorption to the overall subcutaneous absorption of hGH. In lymph-cannulated sheep, average recoveries in peripheral and central lymph were 61.7 and 8.6% of dose, respectively. The lower recovery from central lymph indicates a relevant loss of hGH during transport through the lymphatic system. The mechanistic basis is unclear, as *in vitro* incubation of hGH in fresh central lymph at 37°C showed no loss of hGH over 6 hours.

For PEG-EPOs, Wang and coworkers (2012) demonstrated catabolism to occur in rats both at the subcutaneous administration site as well as in the lymphatics. Subcutaneous bioavailabilities of PEG30-EPO and PEG40-EPO after subcutaneous administration to rats were 38 and 30%, respectively, indicating marked catabolism of a subcutaneous dose during absorption. *In vitro* studies in rat subcutaneous tissue homogenates demonstrated catabolism of PEG-EPOs in subcutaneous tissue, whereas they were stable in both lymph and plasma. Catabolism of PEG-EPOs, however, was also observed in lymph node cell suspensions, providing evidence for catabolism during transport through the lymphatics. The catabolic activity in lymph node cell suspension may be due to phagocytic cells residing in lymph nodes. This study appears to be the first one using *in vitro* experiments to study catabolism after subcutaneous administration.

As described earlier, the subcutaneous bioavailability of IgGs is incomplete and around 60–80% for most marketed IgGs for subcutaneous administration. Experimental data in mice indicate that binding to neonatal Fc receptor (FcRn) influences subcutaneous bioavailability of IgGs. The subcutaneous bioavailability of the murine mAb 7E3 was markedly reduced in FcRn-deficient mice compared with wild-type mice ($28.3 \pm 6.9\%$ vs. $82.5 \pm 15.6\%$) (Wang et al., 2008). Deng and coworkers (2012) showed an increased subcutaneous bioavailability in mice with an IgG variant with increased FcRn affinity at pH 6.0 as compared with wild-type IgG (94.7 vs. 76.3% for wild-type mAb), whereas an IgG variant devoid of FcRn binding had the lowest bioavailability (41.8%). In cynomolgus monkeys, however, mAbs in increased FcRn binding failed to show a clear effect on improved subcutaneous bioavailability (Datta-Mannan et al., 2012). Potential effects of FcRn binding on subcutaneous absorption result from its well known mode of action. Following uptake into cells, IgG binding to FcRn in a pH-dependent manner in the slightly acidic endosomes (pH ca. 6.0) protects IgG from subsequent catabolism in lysosomes. The endosomes fuse with the cell membrane, where the FcRn-bound IgGs are exposed to physiologic pH (pH 7.4). At physiologic pH, IgGs no longer bind to FcRn and are released into the extracellular space. The process protects IgG from catabolism, but may also result in transcytosis (e.g., across the endothelial layer of blood capillaries from the subcutaneous administration site into blood). Thus, during subcutaneous absorption, FcRn binding may prevent IgG from catabolism in subcutaneous tissue/lymphatics or may enhance the FcRn-mediated transcytosis across the vascular endothelium. Based on pharmacokinetic modeling of rituximab subcutaneous absorption in the rat, Kagan and coworkers postulated that both protection of catabolism as well as FcRn-mediated transcytosis into circulation are involved in the FcRn effects on subcutaneous absorption (Kagan et al., 2012; Kagan and Mager, 2013). Deng et al. (2012) postulated the lower subcutaneous bioavailability of mAbs with lower FcRn binding to be due to a lack of protection

at the absorption site. It is of note that a clear relationship between systemic clearance of IgGs and their subcutaneous bioavailabilities was found in a minipig model (Fig. 4) (Zheng et al., 2012). This relationship suggests that the same clearance processes are involved in systemic clearance after intravenous administration and local first-pass catabolism after subcutaneous administration, which is consistent with the observed role of the FcRn-mediated processes after subcutaneous administration and the well known role of FcRn-mediated protection in systemic clearance of IgGs. The minipig as a nonresponder species for most of the IgGs tested is well suited for such correlations, as neither systemic nor local first-pass clearance was influenced by target-mediated drug disposition (TMDD).

TMDD may influence the subcutaneous absorption of biotherapeutics in a responder species, both for IgGs and non-IgGs. If the target is present in the subcutaneous tissue or in the lymphatics, TMDD may add to the first-pass catabolism following subcutaneous administration. This may add to the nonlinear disposition pharmacokinetics frequently observed with biotherapeutics undergoing TMDD. Davis and Bugelski (1998) reported marked dose dependence of subcutaneous bioavailability after administration of an anti-CD4 antibody to a human CD4 transgenic mouse. After a low subcutaneous dose of 0.4 mg/kg to huCD4⁺ mice, no absorption into the systemic circulation was observed, i.e., first-pass catabolism was virtually quantitative. At a high dose of 100 mg/kg, dose-normalized exposure in huCD4⁺ transgenic mice was comparable to exposure in wild-type mice, indicating saturation of target-mediated first-pass catabolism at high dose levels.

Formulation Aspects/Hyaluronidase as Enabling Technology for Subcutaneous Administration

Biotherapeutics for subcutaneous dosing are usually formulated in ready-to-use aqueous buffer solutions that are well tolerated upon injection and ensure stability of the biotherapeutic during the intended shelf life. Upon subcutaneous administration, the formulation is exposed to the physiologic environment of the interstitial space in the subcutaneous tissue, which may lead to changes in pH and ionic composition. Formulation excipients may be removed more rapidly from the injection site as compared with the biotherapeutic. These changes may affect stability and absorption of the administered protein. A detailed summary of such formulation aspects has been provided in a recent review (Kinnunen and Mrsny, 2014) and will not be further discussed here.

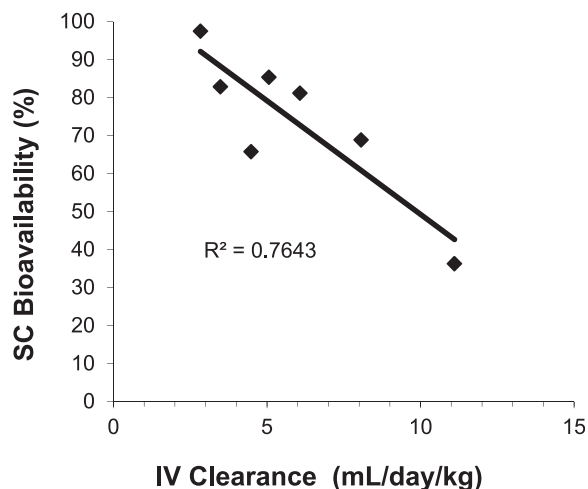


Fig. 4. Correlation between clearance after intravenous (IV) administration and subcutaneous (SC) bioavailability of various mAbs in minipigs. Open symbols represent the reported mean parameter values (data from Zheng et al., 2012).

Subcutaneous injection of biotherapeutics is limited by the volume that can be painlessly injected into the subcutaneous tissue (Jorgensen et al., 1996), which should not exceed 1–2 ml (Hunter, 2008). This limits the total dose of a biotherapeutic that can be administered via the subcutaneous route in a single injection without pain and induration, as the protein concentration in dosing formulation can be increased only to a limited extent. The limited volume is a consequence of the low hydraulic conductivity of the ECM in the subcutis. As described previously, GAGs, particularly hyaluronan, control the hydraulic conductivity. Transient cleavage of hyaluronan in the subcutaneous tissue using animal-derived hyaluronidases has been a well established method to increase hydraulic conductivity and, thus, allow subcutaneous administration of higher volumes and foster subcutaneous spreading and absorption of other drugs (Bookbinder et al., 2006). Recently, a human recombinant hyaluronidase (rHuPH20) has become available (Frost, 2007). Consistent with a transient action and rapid hyaluronan turnover (turnover half-life of 15–20 hours in skin), hyaluronidase effects were found to be reversible within 24 hours in a mouse dye dispersion model (Frost, 2007).

Subcutaneous formulations of trastuzumab and rituximab were developed containing rHuPH20 as a permeation enhancer (Bittner et al., 2012, 2014). The use of rHuPH20 in the formulation allows subcutaneous administration of higher volumes (e.g., 5 ml for the trastuzumab subcutaneous formulation).

Minipigs were selected as nonclinical species of choice for trastuzumab and rituximab subcutaneous formulation testing (Bittner et al., 2012, 2014). The minipig has been demonstrated to be a predictive model for human IgG kinetics after both intravenous and subcutaneous administration (Zheng et al., 2012). The texture of its subcutaneous tissue is considered to be similar to that of humans, with fat lobules separated by a fibrous tissue network connecting dermis and deep fascia/muscle (e.g., in the inguinal area) (Fig. 5). The inguinal area was chosen for subcutaneous formulation testing. It is of note that the structure of the minipig subcutaneous tissues differs across the body (F. Hoffmann-La Roche, data on file). Very pronounced differences were found (e.g., in the lateral thigh) where the subcutaneous tissue is markedly thinner and devoid of fat lobules (Fig. 5). The impact of these site differences on subcutaneous absorption of biotherapeutics remains to be elucidated. Figure 6 shows average serum concentration-time curves of trastuzumab subcutaneous formulations without rHuPH20 or containing 2000 U/ml rHuPH20. Trastuzumab absorption was more rapid from the rHuPH20-containing formulation compared with the control without rHuPH20 (average first-order rate constants 0.828 and 0.166 day⁻¹). The fraction absorbed from compartmental PK analysis was similar across formulations and estimated at 85%. A similar subcutaneous bioavailability was found in humans for the rHuPH20-containing trastuzumab formulation (Table 1).

Kagan and Mager (2013) explored the impact of hyaluronidase on the subcutaneous absorption of rituximab in rats. Hyaluronidase increased both the rate of absorption and the subcutaneous bioavailability of rituximab in the rat model following subcutaneous injection in the back or the abdomen. The hyaluronidase-triggered absorption rate increase may also be used to facilitate a more rapid onset of action of subcutaneous administered biotherapeutics. Coadministration of insulin and hyaluronidase (rHuPH20) has been demonstrated to increase the absorption rate of subcutaneous -administered insulin, which resulted in an improved control of postprandial glucose excursions (Muchmore and Vaughn, 2010).

What Are the Unknowns?

Despite the high relevance of the subcutaneous route for both marketed biotherapeutics and biotherapeutics in development, there are relevant knowledge gaps around subcutaneous absorption of

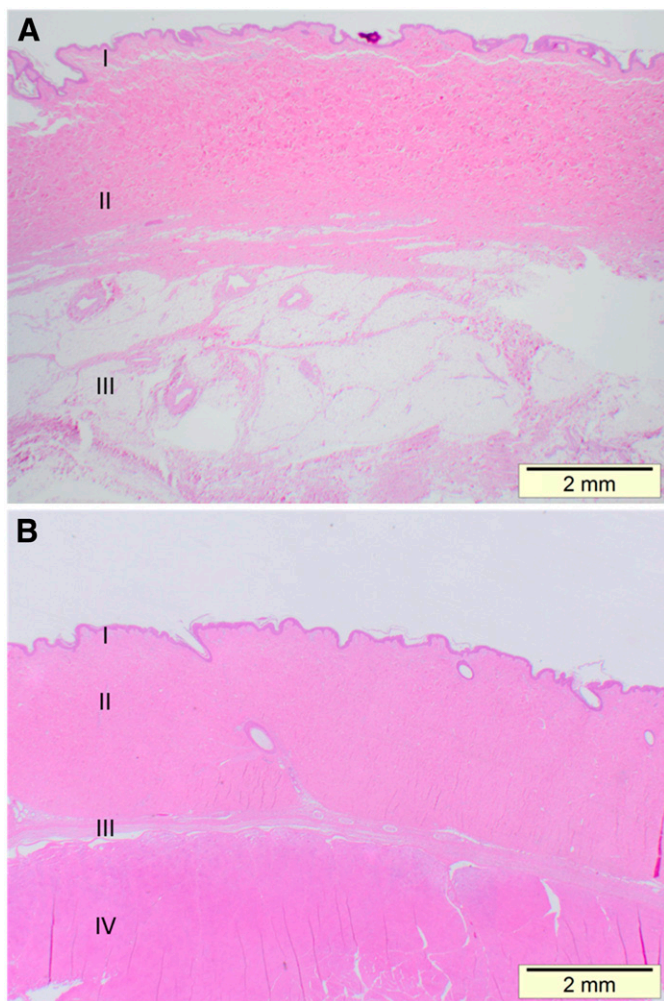


Fig. 5. Sections of skin-muscle tissue from minipigs (15–19 kg) of the inguinal area (A) and the lateral thigh (B) showing, from top to bottom, epidermis (I), dermis (II), subcutis (III), and skeletal muscle [IV; latter missing in (A)]. Hematoxylin-eosin staining. Bar = 2 mm.

biotherapeutics. During the early development of biotherapeutics for subcutaneous administration, animal studies are usually conducted to assess rate and extent of absorption or to assess PK/pharmacodynamic relationships, to translate findings in animals to humans. Subcutaneous bioavailabilities can differ markedly across species (McDonald et al., 2010). Such species differences appear to be more pronounced for non-IgG biotherapeutics. In particular, rodent models often lack predictivity. Thus, for instance, subcutaneous bioavailabilities for insulin-like growth factor 1 are 38–57% and 100% in rats and humans, respectively, whereas for certolizumab pegol, these values are 24–34% and 76–88%, respectively. Similarly, subcutaneous bioavailabilities of interleukin 2 and polyethylene glycol–modified interleukin 2 are markedly lower in furred animals, including rodents, compared with patients, whereas data for domestic pigs were similar to humans (Chen et al., 2000). Differences in presystemic catabolism either in subcutaneous tissue or in draining lymphatics are likely to contribute to these species differences in subcutaneous bioavailability. Species differences in lymphatic uptake or lymph residence time may contribute as well. Further research on presystemic catabolism and lymphatic transport will be needed, including comparison across species. Also, for IgGs, subcutaneous bioavailabilities differ across species. Bioavailability tends to be overestimated in nonhuman primates, whereas for rodents, no clear pattern is evident

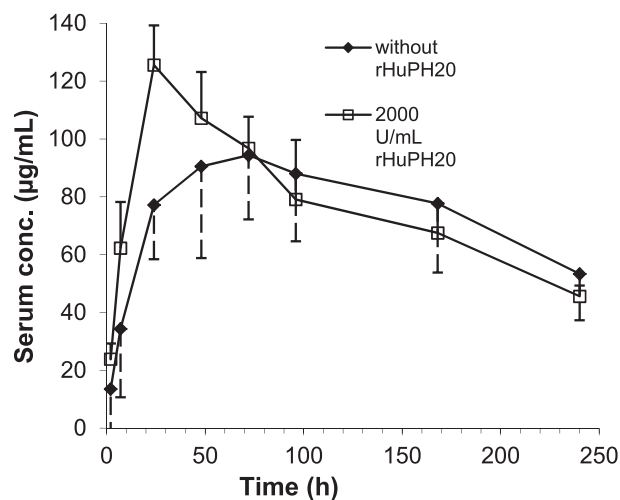


Fig. 6. Average trastuzumab serum concentrations in female minipigs following single subcutaneous administration of trastuzumab in formulations without and with 2000 U/ml rHuPH20: more rapid absorption from rHuPH20-containing formulation (mean \pm S.D., $n = 5$ /dose group) (data from Bittner et al., 2012). Conc., concentration.

(Richter et al., 2012). Minipigs appear to be a promising model for subcutaneous testing of mAbs (Zheng et al., 2012). IgG and Fc-fusion protein bioavailability data provided in Table 1 are consistent with these conclusions. However, care must be exercised when comparing bioavailability figures across compounds and species, as bioavailability figures from standard noncompartmental pharmacokinetic analysis may underestimate the extent of absorption in responder species with relevant TMDD and resulting nonlinear clearance. A more detailed assessment of comparative bioavailability across species, however, is beyond the scope of this minireview. The reasons for those species differences in bioavailability are not understood, and may include species differences in presystemic catabolism combined with species differences in the extent of lymphatic uptake.

Species differences in the rate of subcutaneous absorption have received little attention in the literature. An exception to this is the subcutaneous absorption of erythropoietin, whose absorption rate was shown to scale allometrically with body weight (Woo and Jusko, 2007). In general, subcutaneous absorption of biotherapeutics appears to be more rapid in animals compared with humans. The underlying mechanisms are not understood. As transport in the subcutaneous tissue to the absorbing capillaries appears to be the rate-limiting step in subcutaneous absorption, it is tempting to speculate that species differences in this step are the root cause for more rapid absorption in animals. This may include both smaller distances to be traveled to the next capillary and/or more rapid transport through the ECM of the subcutaneous tissue. A more thorough understanding of species differences in subcutaneous absorption rate and their mechanistic basis would be beneficial for the translation of animal data to humans.

The rate and extent of subcutaneous absorption may also be influenced by the site of injection. Considering interspecies differences in, e.g., subcutaneous tissue structure, translation of injection site differences across species appears to be hardly possible. In animals, site of injection studies are rare. Subcutaneous administration of rituximab at a low dose (1 mg/kg) gave similar bioavailability when administered into the back, abdomen, or foot of rats (Kagan et al., 2012). At a higher dose (10 mg/kg), there was a trend toward higher bioavailability when administered into the abdomen as compared with the back. After administration into the foot, absorption was more rapid as compared with other administration sites. In humans, subcutaneous

absorption of golimumab was found to be similar after administration into the upper arm, abdomen, and thigh (Xu et al., 2010). For other biotherapeutics such as insulin, EPO, and hGH, the subcutaneous absorption differed across administration sites (Bantle et al., 1993; Jensen et al., 1994; Laursen et al., 1994). Future studies may provide a better overall understanding of the influence of various anatomic injection sites on subcutaneous absorption.

Considering the gaps in our mechanistic understanding, PK modeling of subcutaneous absorption relies for the time being on empirical modeling approaches and the use of in vivo data. Thorough knowledge of transport in the ECM and lymphatics as well as catabolic processes and their impact on overall subcutaneous absorption will be required to construct mechanistic PK models of subcutaneous absorption. Also, in vitro models for assessment of subcutaneous absorption are still missing. In the absence of detailed mechanistic understanding, experimental in vivo approaches should be standardized and controlled to reduce potential sources of variability.

Summary

Subcutaneous administration of biotherapeutics offers several potential advantages compared with intravenous administration. Many biotherapeutics, both marketed and in development, are administered via the subcutaneous route. Subcutaneous absorption of biotherapeutics is relatively slow and mostly incomplete. Knowledge of the subcutaneous tissue is important to understand absorption kinetics after subcutaneous administration. Transport in the subcutis appears to be a major contributor to the slow subcutaneous absorption. Larger proteins (>20 kDa) are mostly absorbed via the lymphatic system, although potential species differences are not fully understood yet. Also, the presystemic catabolism leading to incomplete bioavailability is little understood. For IgGs, binding to FcRn increases bioavailability, possibly due to FcRn-mediated protection from catabolism at the injection site and/or draining lymphatics. Overall, several aspects of subcutaneous absorption are still poorly understood, which hampers translation across species. Further research in this area is warranted.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Richter, Jacobsen.

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