Subcutaneous (SC) absorption of biotherapeutics – knowns and unknowns

Wolfgang F. Richter, Björn Jacobsen

Roche Pharmaceutical Research and Early Development, Pharmaceutical Sciences,
Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124,
4070 Basel, Switzerland.
Running Title Page

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Corresponding author: Wolfgang F. Richter
Roche Pharmaceutical Research and Early Development, Pharmaceutical Sciences,
Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Grenzacherstrasse 124,
4070 Basel, Switzerland.

phone       ++41-61-6886215

e-mail       wolfgang.richter@roche.com

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List of non-standard abbreviations:

ECM – extracellular matrix; EPO – erythropoietin; FcRN – neonatal Fc receptor;
GAG – glycosaminoglycan; hGH – human growth hormone; IgG – immunoglobulin G;
IV – intravenous; mAB – monoclonal antibody; SC – subcutaneous; TMDD – target mediated drug disposition
Abstract

Subcutaneous (SC) administration of biotherapeutics offers several potential advantages compared to intravenous (IV) administration. Many biotherapeutics, both marketed or in development, are administered via the SC route. This minireview provides an overview on the presystemic absorption processes following SC administration, the resulting pharmacokinetics after SC administration and provides recent case examples on the development of SC administered drugs with a focus on monoclonal antibodies (mAbs). SC absorption of biotherapeutics is relatively slow and mostly incomplete. Knowledge on the SC tissue is important to understand the absorption kinetics after SC administration. Transport in the subcutis to the absorbing blood or lymph capillaries appears to be a major contributor to the slow SC absorption. Larger proteins (>20 kDa) are mostly absorbed via the lymphatic system, though 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 neonatal Fc receptor (FcRn) is important to obtain a high bioavailability. Overall, several aspects of SC absorption are still poorly understood yet, 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 anti-cancer 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 (SC) administration offers several potential advantages compared to the intravenous (IV) route of administration. While IV infusions usually have to be administered in a hospital or in a doctor’s office, SC 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, SC administration helps to treat patients with poor venous access or to spare patients’ venous capital (Launay-Vacher, 2013). SC administration is of particular benefit for long term or chronic drug treatments. SC administration may be also better tolerated as compared to IV administration, as the slow absorption may abrogate side effects related to high serum concentrations. This was demonstrated for alemtuzumab (Hale 2004). An overview on marketed SC administered monoclonal antibodies (mAb) and immunoglobulin (IgG) fusion proteins is provided in Table 1. SC administration may offer additional advantages for biotherapeutics with short residence time in the body. The slow absorption after SC administration may lead to an absorption rate limited pharmacokinetics and, thus, to a prolonged systemic exposure (Mager and Jusko, 2002). Drawbacks of SC administration include the incomplete bioavailability after SC administration (Richter et al., 2012). The relatively slow SC absorption is also of note, particularly when rapid onset of action is required.
Following IV administration a biotherapeutic is directly injected into the systemic circulation. Following SC administration, however, the biotherapeutic is injected into the extracellular space of the SC tissue, from where it has to be transported to blood or lymph capillaries for absorption, prior to reaching systemic circulation. These processes are influenced by both properties of the biotherapeutic as well as by host factors (Richter et al., 2012). These pre-systemic events have to be considered in understanding the SC administration of biotherapeutics.

The SC 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 on the knowns of presystemic absorption processes following SC administration with a focus on mAbs, the resulting pharmacokinetics after SC administration and provide recent case examples on the development of SC administered drugs. In addition we summarize some of the unknowns in SC absorption of biotherapeutics.

**Presystemic transport processes following SC administration**

SC administration delivers the dosed biotherapeutic into the SC 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 to understand the absorption behavior of biotherapeutics after SC 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 SC 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 Read, 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 like
albumin or IgGs in SC 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 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 re-absorbed into venous capillaries (venules). The re-uptake into blood circulation is driven by the lower pressure in venules as compared to the interstitium. Fluid that was not re-absorbed 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 exsudate is reabsorbed into post-capillary venules (Swartz, 2001), while other views suggest that the lymphatic uptake exceeds re-uptake into venous circulation (Waterhouse et al., 2009).

Convective transport in the interstitium is influenced by size and charge of the molecule. Studies on interstitial transport in dermis of mouse tails showed that convection of dextrans in a molecular weight 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
also below). For very large molecules like 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 co-workers reported that positively charged monoclonal antibodies bind to
rat subcutaneous tissue in vitro in a way that is consistent with electrostatic
interactions (Mach et al., 2011). 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 total interstitial volume for rabbit
subcutaneous tissue (Negrini et al., 2003).

SC absorption is also influenced by host factors. Movement, heat, massage and
other factors can enhance SC 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 occurs usually at the initial lymphatics,
i.e. lymphatic capillaries and pre-collectors. 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 pre-collectors, which form a second plexus at the dermis-subcutis junction. From there lymph drains through lymph collectors in the connective tissue septae of subcutis to the next lymph node. The contribution of the individual parts of lymphatics in dermis and subcutis to the lymphatic absorption of SC administered biotherapeutics remains to be clarified. Thus, it is open 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, by contrast to blood capillaries they allow an easy entry of large molecular weight solutes like 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 in lymph nodes (Aukland and Reed, 1993). About half of the water from lymph may be re-absorbed in blood (Waterhouse et al., 2009), so that the concentrations of solutes in post-nodal lymph may exceed those in interstitial fluid and primary lymph. Post-nodal 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 of the lymphatic system, however, need to be considered. In rats, for instance, the lymphatic drainage routes differ from the one above 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 SC absorption of therapeutic proteins has been extensively studied in a sheep model (Supersaxo et al., 1990; McLennan et al., 2002). Following SC administration the cumulative recovery in peripheral lymph (popliteal lymph node) draining the administration site at a hind leg increased with molecular weight (Figure 1). Starting at molecular weights 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 weight of ca. 40 kDa and above. Lymphatic absorption has been much less studied in other species, so 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 co-workers reported relevant lymphatic recovery of $^{125}$I-labeled pegylated erythropoietin (PEG30-EPO and PEG40-EPO) in lymph from thoracic duct-cannulated rats and dogs (Wang et al., 2012). In rats lymphatic recovery protein-associated radioactivity over 7 days accounted for approximately 60 to 70% of bioavailable material in non-cannulated 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 reported relevant lymphatic recoveries in thoracic duct-cannulated dogs and rats (73 and 27% of dose, respectively) after SC administration of a 48 kDa pegylated peptide (Zou et al., 2013). Considering the observed pre-systemic 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 co-workers reported a low lymphatic recovery from thoracic duct (less than 3% of dose) after SC administration of bovine insulin, bovine serum albumin, and EPO (Kagan et al., 2007). Kojima and co-workers had similar findings for tumor necrosis factor (TNF-\(\alpha\)) in rats (Kojima et al., 1988). It remains to be clarified whether the lymphatic absorption differences in rats reflect a species difference to e.g. sheep or interexperimental variability due to incomplete lymph sampling owing to the different lymphatic system in the rat (see above).

Only few studies on lymphatic absorption of mAbs have been reported. Following SC administration of trastuzumab to thoracic duct-cannulated rats 27% of dose was recovered within 30 h (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 h following SC administration ranged from 10 to 17% of dose, which is equivalent to approximately 25 to 40% of SC bioavailable material in non-cannulated rabbits.

**What determines the slow absorption after SC dosing?**
SC absorption of protein, particularly mABs is slow, as indicated by time to maximum serum concentrations (Tmax) ranging usually from around 3 to up to 8 days in humans (Table 1). Tmax values for mAbs of several days are also observed in larger animals such as cynomolgus monkeys or minipigs (Figure 2; Zheng et al., 2012). Reasons for slow absorption may include slow drug transport through ECM prior to reaching capillaries or a slow lymph flow. Zhao and co-workers developed a hybrid pharmacokinetic (PK) model to describe SC absorption and subsequent disposition (Zhao et al., 2013). Sensitivity analysis demonstrated that only the parameter 'lymph flow' had an impact on Tmax. In the PK model the parameter 'lymph flow' linked the 'interstitial space' compartment of the injection site with compartment 'lymphatic system'. The 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 SC absorption via the lymphatics, as the resistance to fluid and solute transport is higher in the ECM as compared to 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 SC administration site in comparison to a relatively high lymph flow velocity, which supports interstitial
transport as rate limiting step in SC absorption. Wu and co-workers studied the removal of fluorescence-labeled proteins with molecular weights ranging from 23 to 149 kDa (VEGF-C156S (23 kDa), ovalbumin (44.3 kDa), bovine serum albumin (BSA) (66 kDa), and bevacizumab (149 kDa)) from the SC administration site in the mouse footpad (Wu et al., 2012). Half-lives of drug removal from SC administration site were correlated with molecular weight and were as high as 6.81 h for bevacizumab (0.31, 1.57 and 2.85 h for VEGF-C156S, ovalbumin and BSA, respectively) (Figure 3), which both support the hypothesis of removal from SC administration site as rate limiting step. In another study relevant administration site retention was demonstrated also after dorsal SC administration of a fluorescence-labeled immunoglobulin to mice (Filipe et al., 2014). Long residence time of SC administered proteins was also observed in humans. Following SC administration of $^{131}$I-albumin Hollander and co-workers observed an initial loss of radioactivity (10-28% of dose) from the SC dosing site during the first 4 to 8 h after administration in 10 of 15 subjects (Hollander et al., 1961). After the initial loss, radioactivity disappeared from the injection site with a half-life of 33.4 h (range 18-48h). The relatively long residence times at the SC injection sites needs to be compared to residence times in the lymphatic system. A study in the tail microlymphatics of anesthetized mice indicated a median flow velocity of 4.7 µm/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 SC 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 8.9±5.8 cm/min, and the time interval for lymphatic transport from hand to axilla ranged from 3-21 min in 7 subjects (average 9.6±7.2 min). Assuming similar flow rates also in the post-nodal lymphatics, the residence time in the lymphatics can be estimated to be in the order of few hours. With this short estimated lymphatic residence time compared to 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 SC administration.

**Incomplete bioavailability and underlying catabolic processes**

Following SC administration the bioavailability of proteins including IgGs is usually incomplete (Richter et al., 2012; Macdonald et al., 2010). SC bioavailabilities in humans are, for instance, 50%, > 89% and 84% for interferon β-1b, peginterferon α-2a and interferon γ-1b, respectively (data from prescribing information). For marketed IgG the SC bioavailabilities are mostly around 60-80% (Table 1). The incomplete bioavailability may be caused by first pass catabolism at the SC administration site or in the draining lymphatics. Processes of protein first pass catabolism after SC administration are still poorly understood.

Local catabolism at the injection site has been demonstrated, for instance, for insulin. Following SC administration to anesthetized pigs ³H-labeled insulin was removed from the injection site with a half-life of 59 min (Berger et al., 1979). At all sampling times throughout the experiment (up to 160 min post dose), about 20% of radioactivity recovered from the SC injection site was degradation products, indicating a local catabolism of insulin in SC tissue. In rat subcutaneous tissue,
insulin degrading activity was mainly found in the 160000×g supernatant fraction of subcutaneous tissue (Komada et al., 1985). Cathepsin-B and collagenase-like peptidase were detectable as proteolytic enzymes in the rat subcutaneous tissue. Inhibition of proteases in the SC tissue reduces insulin degradation (Takeyama et al., 1991). Pretreatment of the SC injection site with ointments containing the protease inhibitors gabexate mesilate or nafamostat mesilate results in a trend to 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 weight 22 kDa) loss in the lymphatics was demonstrated as the main reason for incomplete SC bioavailability (Charman et al., 2000). In non-cannulated sheep, the SC bioavailability was estimated at 58.4 ± 9.1% (mean ± SEM). Lymph cannulation was done either in the efferent lymph vessel from the popliteal node or in the thoracic duct (peripheral and central lymph collection, respectively). SC bioavailability in lymph cannulated sheep was reduced to ca. 30-40%, indicating a relevant contribution of lymphatic absorption to the overall SC 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 h.

For pegylated erythropoietins (PEG-EPOs) Wang and co-workers demonstrated in rats catabolism to occur both at the SC administration site as well as in the
lymphatics (Wang et al., 2012). SC bioavailabilites of PEG30-EPO and PEG40-EPO after SC administration to rats were 38 and 30%, respectively, indicating marked catabolism of an SC dose during absorption. In vitro studies in rat SC tissue homogenates demonstrated catabolism of PEG-EPOs in SC tissue, while 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 employing in vitro experiments to study catabolism after SC administration.

As described above, also the SC bioavailability of IgGs is incomplete and around 60-80% for most marketed IgGs for SC administration. Experimental data in mice indicate that binding to FcRn influences SC bioavailability of IgGs. The SC bioavailability of the murine mAb 7E3 was markedly reduced in FcRn deficient mice compared to wild-type mice (28.3 ± 6.9% vs. 82.5 ± 15.6%) (Wang et al., 2008). Deng and co-workers showed an increased SC bioavailability in mice with an IgG variant with increased FcRn affinity at pH 6.0 as compared to 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%) (Deng et al., 2012). In cynomolgus monkeys, however, mAbs in increased FcRn binding failed to show a clear effect on improved SC bioavailability (Datta-Mannan et al., 2012). Potential effects of FcRn binding on SC 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 physiological pH (pH 7.4). At physiological pH IgG do no longer bind to FcRn and are released into the extracellular space. The process protects IgG from catabolism, but may result also in transcytosis e.g. across the endothelial layer of blood capillaries from the SC administration site into blood. Thus, during SC absorption FcRn binding may prevent IgG from catabolism in SC tissue/lymphatics or may enhance the FcRn-mediated transcytosis across the vascular endothelium. Based on pharmacokinetic modeling of rituximab SC absorption in the rat, Kagan and co-workers postulated that both protection of catabolism as well as FcRn-mediated transcytosis into circulation are involved in the FcRn effects on SC absorption (Kagan et al., 2012 and 2013). Deng et al. postulated the lower SC bioavailability of mAbs with lower FcRn binding to be due to lack of protection at the absorption site (Deng et al., 2012). It is of note that a clear relationship between systemic clearance of IgGs and their SC bioavailabilities was found in a minipig model (Figure 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 SC administration, which is consistent with the observed role of the FcRn mediated processes after SC administration and the well-known role of FcRn mediated protection in systemic clearance of IgGs. The minipig as a non-responder species for most of the IgGs tested is well suited for such correlations, as both systemic and local first pass clearance are not influenced by target mediated drug disposition (TMDD).

TMDD may influence the SC absorption of biotherapeutics in a responder species, both for IgGs and non-IgGs. If the target is present in the SC tissue or in the
lymphatics TMDD may add to the first pass catabolism following SC administration. This may add to the non-linear disposition pharmacokinetics frequently observed with biotherapeutics undergoing TMDD. Davis and co-workers reported marked dose dependence of SC bioavailability after administration of an anti-CD4 antibody to a human CD4 transgenic mouse (Davis et al., 1998). After a low SC 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, indicating saturation of target mediated first pass catabolism at high dose levels.

Formulation aspects / Hyaluronidase as enabling technology for SC administration

Biotherapeutics for SC 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 SC administration the formulation is exposed to the physiological environment of the interstitial space in the SC tissue, with may lead to changes in pH and ionic composition. Formulation excipients may be removed more rapidly from the injection site as compared to 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.

SC injection of biotherapeutics is limited by the volume that can be painlessly injected into the SC 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 SC route in a single injection without pain and induration, as the protein concentration in dosing formulation can be increased only to a limited extend. 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 SC tissue using animal derived hyaluronidases has been a well-established method to increase hydraulic conductivity and, thus, allow SC administration of higher volumes and foster SC 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 h in skin), hyaluronidase effects were found to be reversible within 24 h in mouse dye dispersion model (Frost, 2007).

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

Minipigs were selected as non-clinical species of choice for trastuzumab and rituximab SC formulation testing (Bittner et al., 2012 and 2014). The minipig has been demonstrated to be a predictive model for human IgG kinetics both after IV and SC administration (Zheng et al., 2012). The texture of its SC tissue is considered to be similar to those of humans, with fat lobules separated by a fibrous tissue network connecting dermis and deep fascia/muscle, e.g. in the inguinal area (Figure 5). The inguinal area was chosen for SC formulation testing. It is of note that the structure of
the minipig SC 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 SC tissue is markedly thinner and devoid of fat lobules (Figure 5). The impact of these sites differences on SC absorption of biotherapeutics remains to be elucidated.

Figure 6 shows average serum concentration-time curves of trastuzumab SC formulations without rHuPH20 or containing 2000 U/mL rHuPH20. Trastuzumab absorption was more rapid from the rHuPH20 containing formulation compared to 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 SC bioavailability was found in humans for the rHuPH20-containing trastuzumab formulation (Table 1).

Kagan and co-workers explored the impact of hyaluronidase on the SC absorption of rituximab in rats (Kagan et al., 2013). Hyaluronidase increased both the rate of absorption and the SC bioavailability of rituximab in the rat model following SC 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 SC administered biotherapeutics. Coadministration of insulin and hyaluronidase (rHuPH20) has been demonstrated to increase the absorption rate of SC administered insulin, which resulted in an improved control of post-prandial glucose excursions (Muchmore and Vaughn, 2010).

**What are the unknowns?**

Despite the high relevance of the SC route for both marketed biotherapeutics and biotherapeutics in development, there are relevant knowledge gaps around SC
absorption of biotherapeutics. During the early development of biotherapeutics for SC administration usually animal studies are conducted to assess rate and extent of absorption or to assess PK/pharmacodynamic relationships, in order to translate findings in animals to humans. SC bioavailabilities can differ markedly across species (McDonald et al., 2010). Such species differences appear to be more pronounced for non-IgG biotherapeutics. Particularly rodent models often lack predictivity. Thus, for instance, SC bioavailabilities for IGF-1 are 38-57% and 100% in rats and humans, respectively, while for certolizumab pegol these values are 24-34% and 76-88%, respectively. Similarly, SC bioavailabilities of IL2 and PEG-IL2 are markedly lower in furred animals including rodents compared to patients, while data in domestic pig were similar to humans (Chen et al., 2000). Differences in pre-systemic catabolism either in SC tissue or in draining lymphatics are likely to contribute to these species differences in SC bioavailability. Species differences in lymphatic uptake or lymph residence time may contribute as well. Further research on pre-systemic catabolism and lymphatic transport will be needed, including its comparison across species. Also for IgGs SC bioavailabilities differ across species. Non-human primates tend to overestimate bioavailability, while for rodents no clear pattern is evident (Richter et al., 2012). Minipigs appear to be a promising model for SC 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 non-linear 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 pre-systemic catabolism combined with species differences in the extent of lymphatic uptake.

Species differences in the rate of SC absorption have received little attention in the literature. An exception to this is the SC absorption of erythropoetin, whose absorption rate was shown to scale allometrically with body weight (Woo et al., 2007).

In general SC absorption of biotherapeutics appears to be more rapid in animals compared to humans. The underlying mechanisms are not understood. As transport in the SC tissue to the absorbing capillaries appears to be the rate limiting step in SC 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 travelled to the next capillary and/or more rapid transport through the ECM of the SC tissue. A more thorough understanding of species differences in SC absorption rate and their mechanistic basis would be beneficial for the translation of animal data to humans.

The rate and extent of SC absorption may be also influenced by the site of injection. Considering interspecies differences in e.g. SC tissue structure, translation of injection site differences across species appears to be hardly possible. In animals site of injection studies are rare. SC administration of rituximab at a low dose (1 mg/kg) gave similar bioavailability when administered into back, abdomen or foot of rats (Kagan et al., 2012). At a higher dose (10 mg/kg) there was a trend to higher bioavailability when administered into abdomen as compared to back. After administration into foot absorption was more rapid as compared to other
administration sites. In humans, SC absorption of golimumab was found to be similar after administration into upper arm, abdomen and thigh (Xu et al., 2010). For other biotherapeutics such as insulin, EPO and hGH the SC 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 on the influence of various anatomical injection sites on SC absorption.

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

**Summary**

SC administration of biotherapeutics offers several potential advantages compared to IV administration. Many biotherapeutics, both marketed or in development, are administered via the SC route. SC absorption of biotherapeutics is relatively slow and mostly incomplete. Knowledge on the SC tissue is important to understand absorption kinetics after SC administration. Transport in the subcutis appears to be a major contributor to the slow SC absorption. Larger proteins (>20 kDa) are mostly absorbed via the lymphatic system, though 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 SC absorption are poorly understood yet, which hampers e.g. translation across species. Further research in this area is warranted.

Authorship contributions:

Wrote or contributed to the writing of the manuscript: Richter WF, Jacobsen B
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Legends for Figures

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

Figure 2: Dose-normalized plasma concentration-time curves of a mAb following subcutaneous (SC) and intravenous (IV) administration to cynomolgus monkeys (mean ± SD, n=3): Slow absorption after SC dosing with maximum concentrations reached after 3-4 days

Figure 3: Half-life of removal from SC administration site after SC administration of fluorescence-labelled vascular endothelial growth factor (VEGF-C156S), ovalbumin (OVA), bovine serum albumin (BSA) and bevacizumab in mice (data from Wu et al., 2012)

Figure 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).

Figure 5: Sections of skin-muscle tissue from minipigs (15-19 kg) of (A) the inguinal area and (B) the lateral thigh 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
Figure 6: Average trastuzumab serum concentrations in female minipigs following single subcutaneous administration of trastuzumab in formulations without and with 2000 U/mL recombinant human hyaluronidase (rHuPH20): More rapid absorption from rHuPH20 containing formulation (mean ± SD, n = 5/dose group) (data from Bittner et al., 2012).
Table 1: Overview on marketed IgGs and IgG Fc-fusionproteins administered via the SC route

<table>
<thead>
<tr>
<th>INN name</th>
<th>Tradename</th>
<th>Antibody type</th>
<th>Target</th>
<th>Tmax in humans (days)</th>
<th>Bioavailability in humans and animals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adalimumab</td>
<td>Humira</td>
<td>IgG1</td>
<td>TNF</td>
<td>5.5</td>
<td>Humans: 64 Cynomolgus mk: 96</td>
</tr>
<tr>
<td>Canakinumab</td>
<td>Ilaris</td>
<td>IgG1</td>
<td>IL-1β</td>
<td>7</td>
<td>Humans: 70 Marmoset: 60</td>
</tr>
<tr>
<td>Denosumab</td>
<td>Prolia</td>
<td>IgG2</td>
<td>RANK-L</td>
<td>10</td>
<td>Humans: 78 Mice: 86 Cynomolgus mk: 28-100</td>
</tr>
<tr>
<td>Golimumab</td>
<td>Simponi</td>
<td>IgG1</td>
<td>TNF</td>
<td>2-6</td>
<td>Humans: 53 Cynomolgus mk: 77</td>
</tr>
<tr>
<td>Omalizumab</td>
<td>Xolair</td>
<td>IgG1</td>
<td>IgE</td>
<td>7-8</td>
<td>Humans: 62 Mice: 90 Cynomolgus mk: 64-104</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Mabthera</td>
<td>IgG1</td>
<td>CD20</td>
<td>3</td>
<td>Humans: 71 Mice: 63 Minipig: 71</td>
</tr>
<tr>
<td>Tocilizumab</td>
<td>Actemra /</td>
<td>IgG1</td>
<td>IL-6R</td>
<td>-</td>
<td>Humans: 80 Minipig: 84 Cynomolgus mk: 72</td>
</tr>
<tr>
<td></td>
<td>RoActemra</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Herceptin</td>
<td>IgG1</td>
<td>HER2</td>
<td>3</td>
<td>Humans: 82 Mice: 83 Minipigs: 82 Cynomolgus mk: ca. 100</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>Stelara</td>
<td>IgG1</td>
<td>IL-12/23p40</td>
<td>8.5</td>
<td>Humans: 57 (24-95) Cynomolgus mk: 97</td>
</tr>
<tr>
<td>Abatacept</td>
<td>Orenzia</td>
<td>Fc-fusionprotein</td>
<td>CD80/86</td>
<td>3-5</td>
<td>Humans: 79 Mice: 78-110 Rat: 41-63</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Enbrel</td>
<td>Fc-fusionprotein</td>
<td>TNF</td>
<td>3</td>
<td>Humans: 76 Mice: 58 Cynomolgus mk: 73</td>
</tr>
<tr>
<td>Rilonacept</td>
<td>Arcalyst</td>
<td>Fc-fusionprotein</td>
<td>IL-1α, IL-1β, IL-1ra</td>
<td>-</td>
<td>Humans: 43 Mice: 78 Rats: 54 Cynomolgus mk: 70</td>
</tr>
<tr>
<td>Romiplostim</td>
<td>Nplate</td>
<td>Fc-fusionprotein</td>
<td>Thrombopoietin receptor</td>
<td>0.6</td>
<td>Humans: - Rats: 21-28 Cynomolgus mk: 19 Rhesus mk: 45-74</td>
</tr>
</tbody>
</table>

a: Data on time to maximum serum concentration (Tmax) and bioavailability collected from EMA Summary of Product Characteristics (http://www.ema.europa.eu/ema/index.jsp?curl=pages/home/Home_Page.jsp&mid=), EMA assessment reports or the FDA homepage (http://www.accessdata.fda.gov/scripts/cder/drugsatfda

b: Bioavailability increases with increasing dose
Figure 3

Absorption T1/2 (h)

Molecular weight (kDa)

- VEGF-C156S
- OVA
- BSA
- Bevacizumab
- R² = 0.9972

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Figure 4

R² = 0.7643
Figure 5
Figure 6

- without rHuPH20
- 2000 U/mL rHuPH20

Serum conc. (µg/mL) vs. Time (h)

0 20 40 60 80 100 120 140
0 50 100 150 200 250

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