Pharmacokinetic Modeling of the Subcutaneous Absorption of Therapeutic Proteins

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ABSTRACT

Subcutaneous injection is an important route of administration for therapeutic proteins that provides several advantages over other modes of parenteral delivery. Despite extensive clinical use, the exact mechanism underlying subcutaneous absorption of proteins is not completely understood, and the accuracy of prediction of absorption of biotherapeutics in humans remains unsatisfactory. This review summarizes a variety of models that have been developed for describing the pharmacokinetics of therapeutic proteins administered by subcutaneous injection, including single- and dual-pathway absorption models. Modeling of the lymphatic uptake and redistribution, absorption of monoclonal antibodies and insulin, and population analysis of protein absorption are discussed. The review also addresses interspecies modeling and prediction of absorption in humans, highlights important factors affecting the absorption processes, and suggests approaches for future development of mechanism-based absorption models.

Introduction

Biotherapeutics denotes a rapidly growing class of drugs with more than 200 biopharmaceuticals already approved, including vaccines, hormones, cytokines, and monoclonal antibodies (mAbs) (Walsh, 2010; Janowitz, 2011; Reichert, 2012). Several hundred new products are under development, such as a variety of mAbs, Fc-fusion proteins, antibody-drug conjugates, and alternative protein scaffolds (Beck et al., 2010; Dostalek et al., 2013). Parenteral administration is a major limitation for treatment with biotherapeutics. The subcutaneous route may be advantageous over intravenous or intramuscular administration, especially for improving patients’ convenience and reducing treatment costs (McDonald et al., 2010; Dychter et al., 2012; Richter et al., 2012). Subcutaneous injection has been used clinically for decades for administration of drugs such as insulin, erythropoietin, and growth hormone. Several mAbs and Fc-fusion proteins are currently approved for subcutaneous administration, and the interest in subcutaneous delivery of other antibody-based treatments is growing (Mishbah et al., 2009; Aue et al., 2010; Bittner et al., 2012; Bittner and Schmidt, 2012; Davies et al., 2014; Tobinai, 2014). However, variable and incomplete bioavailability is a major concern for subcutaneous delivery, and the mechanisms of subcutaneous absorption of protein therapeutics are not fully understood and require further investigation. Furthermore, current methods for predicting absorption of biotherapeutics in humans are inadequate (McDonald et al., 2010; Richter et al., 2012). Pharmacokinetics of protein therapeutics is often nonlinear, and extensive mathematical modeling may be required for resolving complexities associated with their absorption and disposition. This review summarizes a variety of existing models for describing the pharmacokinetics of therapeutic proteins administered by subcutaneous injection, highlights important factors affecting the absorption process, and suggests approaches for future development of mechanism-based absorption models.

Background

Subcutaneous injection delivers drugs into the hypodermis, the intersitial space located below the dermis. The structure and physiology of the interstitial space have been reviewed extensively (Porter and Charman, 2000; Swartz, 2001). After injection, proteins administered by subcutaneous injection, including single- and dual-pathway absorption models. Modeling of the lymphatic uptake and redistribution, absorption of monoclonal antibodies and insulin, and population analysis of protein absorption are discussed. The review also addresses interspecies modeling and prediction of absorption in humans, highlights important factors affecting the absorption processes, and suggests approaches for future development of mechanism-based absorption models.
tight junctions are absent (Swartz, 2001). The structure of the lymphatic system allows for unidirectional flow; and the lymph is formed as the interstitial fluid enters the lymphatic capillaries, driven by the gradient in the hydrostatic and osmotic pressure. The role of the lymphatic system in subcutaneous absorption of therapeutic proteins and particulate formulations has been investigated in several species (Porter et al., 2001; McLennan et al., 2005b; Gershkovich et al., 2007; Wang et al., 2012).

Factors affecting the absorption of biotherapeutics after subcutaneous injection can be divided into species, subject, and drug dependent. Differences in skin morphology, such as presence of panniculus carnosus muscle in lower species and the greater mobility of skin in scrub animals, may contribute to the lack of correlation between the bioavailability of biotherapeutics in animals and humans (McDonald et al., 2010; Richter et al., 2012). Differences in binding affinity between Fc receptor of neonates (FcRn) and IgG among species may complicate interspecies modeling of subcutaneous absorption of mAbs (Ober et al., 2001). Although pig skin is similar to human skin, the rate of subcutaneous absorption of several mAbs was 2- to 5-fold greater in minipigs as compared with humans (Zheng et al., 2012). The bioavailability of mAbs in humans is often overestimated based on nonhuman primate data (Richter et al., 2012). The structure of the subcutaneous tissue in animals and humans varies depending on the anatomic location in the body, which may result in different absorption behavior and increase the pharmacokinetic variability (Beshyay et al., 1991; Macdougall et al., 1991; Ter Braak et al., 1996; Kota et al., 2007; Kagan et al., 2012). In humans, hypodermis thickness increases with body weight, decreases with age, and depends on gender (Gibney et al., 2010). For example, the distance between the skin and the gluteal muscle was 1.6 cm in normal women (body mass index 18.5–24.9 kg/m²) as compared with 3.6 cm in obese women (body mass index 30–40 kg/m²) (Shah et al., 2014). Other factors controlling the rate and the extent of absorption include the molecular size of the drug, formulation excipients, restraint status or anesthesia, and application of heat or pressure (Porter and Charman, 2000; Porter et al., 2001; McLennan et al., 2005b). For example, the rate of the thoracic lymph flow was higher in freely moving animals as compared with anesthetized ones (Lindena et al., 1986), and the rate of absorption of soluble insulin was positively correlated with the subcutaneous blood flow (Vora et al., 1992).

**Modeling of the Subcutaneous Absorption of Therapeutics Proteins**

Pharmacokinetic and pharmacodynamic relationships for therapeutic proteins tend to be more complex than for small molecule drugs. High-affinity binding to pharmacologic targets (soluble and tissue bound) often leads to nonlinear distribution and elimination patterns, which are dependent on the drug dose and the underlying dynamics of the target. The biodisposition of mAbs is affected by multiple binding interactions specific to the particular drug (through the Fab region) or drug class (through the Fc region). Noncompartmental methods of analysis, including calculation of the bioavailability and interspecies scaling, can be unreliable for biotherapeutics due to dose-dependent pharmacokinetics and slow equilibration between the plasma and the sites of elimination (which violates the underlying assumptions of this approach). Therefore, advanced mechanism-based pharmacokinetic/pharmacodynamic models may be required for describing absorption, biodisposition, and efficacy in preclinical and clinical studies.

Significant progress has been made in understanding the complexities of the systemic pharmacokinetics of protein therapeutics in recent years. Target-mediated (or receptor-mediated) drug disposition models are often applied to mechanistically describe the systemic disposition of biotherapeutics (Levy, 1994; Mager and Jusko, 2001). Binding of mAbs to FcRn and the role of this interaction in protecting mAbs from degradation and extending their biologic half-life is well established (Ghetie et al., 1996; Junghans and Anderson, 1996), and an endosomal recycling model was proposed to describe this phenomenon (Hansen and Balthasar, 2003).

This review is focused on the absorption component of the pharmacokinetic models developed for therapeutic proteins. In the majority of the discussed studies, modeling of systemic disposition was supported by data obtained after intravenous administration of compounds. It should be noted that the selection of the systemic structural model (especially in the case of nonlinear disposition) might have a substantial impact on the fit of the absorption phase of pharmacokinetic profiles. Analysis of such interactions and their impact on the selection of appropriate absorption models is beyond the scope of this review. To facilitate the discussion, equations describing the systemic disposition were replaced by a (± disposition) term. For the purposes of this review, absorption models were divided into single- and dual-pathway categories. The later was subdivided into empirical models (based on studies measuring plasma/serum concentrations only) and mechanistic models describing the plasma pharmacokinetic profiles and the amount of drugs in the lymph simultaneously. Absorption models for mAbs and insulin are reviewed separately.

**Single-Pathway Subcutaneous Absorption Models**

Although subcutaneous absorption of therapeutic proteins is controlled by multiple factors, a rate-limiting step in the absorption pathway (such as transport through the interstitium, penetration of blood capillaries, or lymph flow rate) may limit our ability to identify these complexities. The simplest structural model being evaluated to describe the subcutaneous absorption includes a first-order absorption rate constant (k₀) and a bioavailability term (F) (Fig. 1A). This model has been successfully applied for describing the subcutaneous pharmacokinetics of many proteins—such as human interferon α2b (IFN-α2b) in healthy human volunteers (Radwanski et al., 1987) and recombinant human growth hormone in rhesus monkeys (Sun et al., 1999). Selection of this simple model is often dictated by limited data (e.g., single-dose level or sparse sampling during the absorption phase). The number the model parameters can be further reduced by estimating the apparent volume of distribution (Vd/F) and clearance (CL/F) terms if the intravenous administration is not evaluated and a priory knowledge of the systemic absorption rate constants; τ, duration of the zero-order absorption; Tlag, a lag time for the first-order absorption; Vmax and Kp, parameters for a Michaelis-Menten kinetics.
bioavailability is unavailable. Absorption rate-limited elimination (flip-flop kinetics) should be considered in this case, and physiologic interpretation of the modeling results may be problematic. When the rates of absorption and elimination appear similar, the corresponding rate constants can be constrained to be equal. This approach was applied to describe the time-course of IFN-α2a and pegylated (PEG)-IFN-α2a concentrations after subcutaneous administration of a range of doses (3–18 MIU) to healthy volunteers (Nieforth et al., 1996) and PEG-IFN-β1a (0.3–3.0 MIU/kg) to cynomolgus monkeys (Mager et al., 2005).

A Michaelis-Menten model ($V_{\text{max}}$ and $K_m$) can be implemented if saturation of the absorption kinetics with increasing dose level are observed. This mechanism was used to describe the absorption of recombinant human growth hormone in chronic dialysis patients and healthy subjects (Klitgaard et al., 2009) and to support dose selection for exenatide in clinical studies (Fineman et al., 2007). Carrier-mediated transport or precipitation at the injection site are commonly postulated; however, the nature of this saturable absorption for proteins is generally unknown.

A model with a single transit compartment between the injection site and the central compartment of the systemic disposition model was proposed to describe a distinct delay in subcutaneous absorption of some proteins (Fig. 1B) (Mager and Jusko, 2002; Mager et al., 2003; Segrave et al., 2004). The transit compartment was sometimes attributed to the lymphatic system, although no direct assessment of the lymphatic component was performed. Parameterization of the model includes the bioavailability ($F$) and two first-order rate constants ($k_{a1}$ and $k_{a2}$), which cannot be uniquely identified.

Biotherapeutics often exhibit nonlinear subcutaneous absorption, and dose-dependent parameters are commonly used to facilitate model fitting. Although this approach may be appropriate in fit-for-purpose scenarios, it provides little insight into the mechanisms of absorption for a specific drug, which is essential for accurate assessment of the bioavailability, optimizing delivery, and predicting absorption in humans, including the effects of population covariates. For example, a dose-dependent absorption rate constant ($k_{a2}$, increasing with the dose level) was required for describing the absorption of leukemia inhibitory factor administered to sheep (Segrave et al., 2004). Evaluation of other absorption models, such as zero-order and a combination of the first-order and zero-order, did not provide satisfactory results. Dose-dependent bioavailability (increasing with the dose level) combined with target-mediated systemic disposition was used to capture the pharmacokinetics of IFN-β1a in cynomolgus monkeys (Mager et al., 2003). Saturation of the injection site-specific metabolism was hypothesized to be the source of this phenomenon; however, incorporation of the Michaelis-Menten mechanism did not improve the fit (Mager et al., 2003).

### Empirical Dual-Pathways Subcutaneous Absorption Models

The existence of two absorption pathways (through the blood and lymph capillaries) for large molecules after injection into the interstitial space has been recognized for many years. Deconvolution analysis of plasma concentration-time profiles after subcutaneous administration frequently demonstrated several absorption phases for therapeutic proteins (Radwanski et al., 1998; Ramakrishnan et al., 2004). A model with two parallel absorption processes was suggested, although a direct assessment of the contribution of the lymphatic system was not performed experimentally. A general schematic of the model is shown in Fig. 1C, and several variations of this model have been used to describe the absorption of proteins in animals and humans (Table 1). The model uses a combination of a zero- and first-order processes originating from the subcutaneous space and leading to the central distribution compartment. The zero-order process is limited in duration from the time of injection to time $\tau$, and is usually described as absorption through the blood capillaries. The first-order process ($k_a$) is usually attributed to lymphatic absorption that occurs after a delay ($T_{\text{lag}}$). The fractions delivered by the first- and zero-order processes are modeled as parameters $F_{rc}$ and $(1-F_{rc})$. In a general form the resulting input function can be represented by the following equation:

$$\text{Input} = \frac{F \cdot \text{Dose} \cdot (1 - F_{rc})}{\tau} \cdot \Theta(\tau - t) + k_a \cdot F \cdot \text{Dose} \cdot F_{rc} \cdot e^{-k_a(t-T_{\text{lag}})} \cdot \Theta(t - T_{\text{lag}})$$

where $F$ is the absolute bioavailability, and $\Theta(x)$ is the step function that equals to 1 for $x > 0$ and 0 otherwise. To further delay the absorption, a single transit compartment is sometimes added to either one of the two pathways with sequential first-order uptake into the central compartment ($k_{a2}$ or $k_{a3}$, dashed-line compartments in Fig. 1C) (Bocci et al., 1986; Radwanski et al., 1998). To reduce the number of the estimated parameters, $T_{\text{lag}}$ and $\tau$ are often constrained to be equal to each other (Table 1). However, this assumption essentially transforms the model into a sequential two-phase absorption, which limits its physiologic interpretation. It should be noted that by fixing the fraction absorbed of one of the processes to zero the model could be reduced to a single-pathway model (which can be useful for model evaluation).

This model was suggested to capture the pharmacokinetics of recombinant human IFN-α2a after subcutaneous injection to rabbits (Bocci et al., 1986). The systemic disposition was described using three compartments and linear elimination. Separate sets of parameters were estimated for IFN-α2 given alone or in combination with absorption modifiers (albumin and hyaluronidase). Individual pharmacokinetic profiles of interleukin-10 in healthy humans after intravenous and subcutaneous dosing were described by a similar model (Radwanski et al., 1998). A single transit compartment with a first-order rate transfer to the systemic circulation ($k_{a2}$) was added to the linear pathway to further delay the absorption. The mean duration of the zero-order absorption ($\tau$) was estimated as 0.47 ± 0.52 hour (mean ± S.D.), and the $T_{\text{lag}}$ for the first-order absorption was 0.23 ± 0.52 hour, which allowed for two processes to occur simultaneously. Similar to the single-pathway models, dose-specific parameters (bioavailability and first-order absorption rate constant) have been used to account for nonlinearities in the absorption process (Ramakrishnan et al., 2003, 2004).

A wide range of values was reported for the parameters for various proteins in different species (Table 1). Although this model was originally proposed to mechanistically describe protein absorption, limited inferences of physiologic relevance of the estimated parameters could be drawn at this time. For example, the fraction of the bioavailable dose absorbed through the zero-order pathway varied from 5 to 88%, and the estimated duration of the zero-order process in humans ranged from 0.47 to 60 hours.

### Mechanistic Dual-Pathway Subcutaneous Absorption Models (Models of Lymphatic Absorption)

Following an early work that found a direct linear correlation between the molecular weight of compounds and the percentage of the subcutaneous dose recovered in peripheral lymph (Supersaxo et al., 1990), other investigators evaluated the contribution of the lymphatic pathway to the subcutaneous bioavailability of protein drugs in a sheep model. These studies provided most of the data for development of the lymphatic absorption models (McLennan et al., 2003, 2005a, 2006; Kota et al., 2007). The first generation of this model included
TABLE 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species/Population</th>
<th>Duration of the Zero-Order Process</th>
<th>Rate Constant for the Zero-Order Process</th>
<th>Bioavailability</th>
<th>Percentage of Bioavailable</th>
<th>Rate Constant for the First-Order Process</th>
<th>Systemic Disposition Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra</td>
<td>Rat</td>
<td>2 h</td>
<td>0.39 h⁻¹</td>
<td>100%</td>
<td>2CM with linear elimination</td>
<td>0.39h⁻¹</td>
<td>2CM with linear elimination</td>
<td>Liu et al., 2011</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Rat</td>
<td>10 h</td>
<td>0.36 h⁻¹</td>
<td>50%</td>
<td>2CM with linear and MM elimination</td>
<td>0.36h⁻¹</td>
<td>2CM with linear elimination</td>
<td>Woo et al., 2006</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Rat</td>
<td>13.5 h</td>
<td>0.37 h⁻¹</td>
<td>35%</td>
<td>2CM with linear and MM elimination</td>
<td>0.37h⁻¹</td>
<td>2CM with linear elimination</td>
<td>Ramakrishnan et al., 2004</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Cynomolgus monkey</td>
<td>10 h</td>
<td>0.044-0.053 h⁻¹</td>
<td>90%</td>
<td>2CM with linear elimination</td>
<td>0.044-0.053 h⁻¹</td>
<td>2CM with linear elimination</td>
<td>Kozlowski et al., 2009</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>6.6 h</td>
<td>0.0146 h⁻¹</td>
<td>98%</td>
<td>1CM with linear elimination</td>
<td>0.0146 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Abraham et al., 2009</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.47 h</td>
<td>0.3884 + 0.0002495 h⁻¹</td>
<td>100%</td>
<td>1CM with MM elimination</td>
<td>0.3884 + 0.0002495 h⁻¹</td>
<td>1CM with MM elimination</td>
<td>Liu et al., 2011</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.27 h</td>
<td>0.16 h⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.16 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Woo et al., 2006</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.146 h</td>
<td>0.61 h⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.61 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Ramakrishnan et al., 2003</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.064 h</td>
<td>0.146 h⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.146 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Ramakrishnan et al., 2003</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.046 h</td>
<td>0.7 day⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.7 day⁻¹</td>
<td>1CM with linear elimination</td>
<td>Ramakrishnan et al., 2003</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.0146 h</td>
<td>0.403 h⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.403 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Ramakrishnan et al., 2003</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>Human patients</td>
<td>5 min</td>
<td>0.018 h⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.018 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Abraham et al., 2009</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>Rat</td>
<td>2 min</td>
<td>0.0018 h⁻¹</td>
<td>100%</td>
<td>1CM with linear elimination</td>
<td>0.0018 h⁻¹</td>
<td>1CM with linear elimination</td>
<td>Abraham et al., 2009</td>
</tr>
</tbody>
</table>

Note: Additional transit compartment for the first-order process.

PK Modeling of Subcutaneous Absorption of Biotherapeutics

![Fig. 2](image-url)

**Fig. 2.** Schematic of the first-generation mechanistic dual-pathway subcutaneous (SC) absorption models. (A) Model without a lymph compartment was used for leptin (McLennan et al., 2003) and darbepoetin (McLennan et al., 2006) in sheep. (B) Model with a separate lymph compartment was used for erythropoietin (McLennan et al., 2005a) and darbepoetin (Kota et al., 2007) in sheep. The model is fitted simultaneously to the plasma (or serum) pharmacokinetic profiles in control (noncannulated) and lymph-cannulated animals and to the cumulative recovery in peripheral or central lymph of lymph-cannulated animals. Systemic disposition can be described by different models depending on the drug and, for simplicity, is shown as a single gray compartment. Here, $k_{blood}$, $k_{lymph}$, and $k_{loss}$ are the first-order rate constants for uptake by blood and lymph capillaries, and degradation; $F_b$, $F_{lymph}$, and $F_{blood}$ represent the fraction of bioavailable drug absorbed by the corresponding pathway.

The cumulative absorption process can be described by the following equations:

1. $F_b = \frac{k_{blood}}{k_{blood} + k_{lymph} + k_{loss}}$ (2)
2. $F_{lymph} = \frac{k_{lymph}}{k_{blood} + k_{lymph}}$ (3)
3. $F_{blood} = \frac{k_{blood}}{k_{blood} + k_{lymph}}$ (4)

where $k_{blood}$, $k_{lymph}$, and $k_{loss}$ are the first-order rate constants for uptake by blood and lymph capillaries, and degradation; $F_b$ is the absolute bioavailability, and $F_{blood}$ and $F_{lymph}$ represent the fraction of bioavailable drug absorbed by the corresponding pathway.
amount of leptin in the peripheral lymph was 34.4 ± 9.7% of the
dose, and the area under the plasma concentration-time curve
(AUC) in the lymph-cannulated group was 38% of the AUC of the
noncannulated group. Systemic pharmacokinetics were described
using a two-compartment model with linear elimination (based on
0.1 mg/kg i.v. dose), and the corresponding disposition param-
eters were fixed for analysis of subcutaneous groups. Absorption
was modeled as two first-order processes originating from the
injection space ($k_{\text{blood}}$ and $k_{\text{lymph}}$) (Fig. 2A). Individual animal
data were described using the following equations:

$$\frac{dSC}{dt} = -k_a \cdot SC$$ (5)

For the control group:

$$\frac{dC_{\text{noncan}}}{dt} = \frac{k_a \cdot SC \cdot F}{V_c} + \text{disposition}$$ (6)

For the lymph-cannulated group:

$$\frac{dC_{\text{can}}}{dt} = \frac{k_a \cdot SC \cdot F \cdot (1 - F_{\text{lymph}})}{V_c} + \text{disposition}$$ (7)

$$\frac{dL}{dt} = k_a \cdot SC \cdot F_{\text{abs}} \cdot F_{\text{lymph}}$$ (8)

where $SC$ is amount of the drug at the injection site, $C$ is the
concentration in the central compartment (with the volume of $V_c$),
and $L$ is the amount of the drug in the lymph of the cannulated
group. The absorption rate constant ($k_a = k_{\text{blood}} + k_{\text{lymph}}$) was allowed to vary
between lymph-cannulated and control animals.

The model was also applied to describe the absorption of
darbepoetin, a hyperglycosylated analog of recombinant human eryth-
ropoietin $\alpha$, in sheep after subcutaneous administration of 2 $\mu$g/kg (at
the interdigital space of hind leg) to control and peripheral lymph-
cannulated animals (McLennan et al., 2006). Mean data from all
groups were fitted simultaneously, and the absorption parameters were
shared between subcutaneous groups; however, a separate elimination
rate constant was estimated for the intravenous and each of the
subcutaneous groups.

A model with a separate lymphatic compartment (Fig. 2B) was used
to describe the absorption of recombinant human erythropoietin $\alpha$ after
subcutaneous injection to sheep (McLennan et al., 2005a). Nonlinearity
in systemic disposition (10–1000 IU/kg i.v. dose) was described using
a combination of a linear and Michaelis-Menten elimination
mechanisms. A single subcutaneous dose (400 IU/kg, interdigital
space of hind leg) was administered to control, peripheral lymph-
cannulated (popliteal duct), and central lymph-cannulated (thoracic
duct) animals. Mean data from all groups were fitted simultaneously,
and all parameters were estimated with good precision. The rates of
absorption through the blood capillaries and the rate of presystemic
degradation were described using the first-order rate constants ($k_{\text{blood}}$
and $k_{\text{loss}}$). The rate of lymphatic uptake ($k_{\text{lymph}}$) was shared between
all groups; however, a lag time parameter (0.52 hour) was estimated
to account for a delay in erythropoietin appearance in the central
lymph. It is unclear whether the lag time was included for the SC
control group. Another first-order rate constant was estimated to
describe the drug transfer from the lymph compartment to the central
compartment ($k_{\text{input}}$) (Fig. 2B):

$$\frac{dSC}{dt} = -(k_{\text{blood}} + k_{\text{lymph}} + k_{\text{loss}}) \cdot SC$$ (9)

For the control group:

$$\frac{dC_{\text{noncan}}}{dt} = \frac{k_{\text{blood}} \cdot SC}{V_c} + \frac{k_{\text{input}} \cdot L}{V_c} + \text{disposition}$$ (10)

$$\frac{dL_{\text{noncan}}}{dt} = k_{\text{lymph}} \cdot SC - k_{\text{input}} \cdot L$$ (11)

For the lymph-cannulated group:

$$\frac{dC_{\text{can}}}{dt} = \frac{k_{\text{blood}} \cdot SC}{V_c} + \text{disposition}$$ (12)

$$\frac{dL_{\text{can}}}{dt} = k_{\text{lymph}} \cdot SC$$ (13)

A similar absorption model structure was used to describe the
pharmacokinetics of darbepoetin in another study that compared
lymphatic uptake from different anatomic locations (Kota et al.,
2007). Central lymph was collected in all cannulated groups.
Parameters for the systemic disposition were shared among all groups.
The absorption model for the injection at the interdigital space
followed the model for erythropoietin (eqs. 9–13; Fig. 2B).
Absorption from the abdominal injection site followed a biphasic
pattern that was captured by two first-order inputs for both lymph and
blood pathways. Specific equations used by the investigators were
not provided, and it is unclear whether the second rate constant was added
to or replaced the first rate constant after the lag time. The presystemic
degradation rate constant ($k_{\text{loss}}$) was constrained to be equal between
noncannulated and lymph-cannulated groups.

A common limitation of the previously discussed lymphatic ab-
sorption models is the inability to account for redistribution of sys-
temically available protein to the lymphatic system. The drug collected in
the lymphatic fluid after subcutaneous injection comes from two sources:
1) drug entering the lymphatics at the site of injection and 2) systemically
available drug that extravasated and was drained from the tissues by the
lymphatic system (Fig. 3A) (Kagan et al., 2007). The role of the
lymphatic system in the recovery of macromolecules from the interstitial
space in tissues and return to the systemic circulation is well established.
Several decades ago, this mechanism was incorporated into the physi-
ologically based models describing the pharmacokinetics of antibodies
and antibody fragments (Covell et al., 1986; Jain and Baxter, 1988;
Baxter et al., 1994, 1995). Quantitative information on the lymphatic
recovery of macromolecules after redistribution of the systemically
available drug is limited. Collection of the lymph after the in-
travenous administration of compounds can be used to obtain such
data. For example, plasma and central lymph concentration-time
profiles after intravenous administration of tumor necrosis factor,
IFN-β, IFN-α2, and interleukin-2 to rabbits (Bocci et al., 1987,
1988a,b, 1990) and PEG40-erythropoietin to a dog (Wang et al.,
2012) have been reported. The recovery of IFN-α2a in peripheral
lymph was 0.001–0.003% of the intravenous dose in sheep (Supersaxo
et al., 1988). Similar concentrations of erythropoietin were found in
the plasma and thoracic lymph 1.5 hours after intravenous injection to rats
(Kagan et al., 2007). Up to 20% of the dose of a radiolabeled peptide
(15HJMR)-1 was recovered in thoracic lymph over 3 days after
intravenous injection to dogs (Zou et al., 2013), and 44% of trastuzumab
intravenous dose was collected over 30 hours in the thoracic lymph of
rats (Dahlberg et al., 2014).

Collectively, these works indicate that redistribution of the sys-
temically available drug to the lymph can be significant, so including
this pathway(s) into the pharmacokinetic model is important. A failure to account for this phenomenon might result in an overestimation of the lymphatic absorption, especially if central lymph is collected and the total collection period spans a long time. Comparable concentrations measured in the plasma and thoracic lymph after subcutaneous administration of several proteins to rats was attributed to the redistribution phenomenon, and including this pathway into the absorption model was suggested (Kagan et al., 2007); however, the estimation of the corresponding parameters was not performed. Recently, Dahlberg et al. (2014) extensively evaluated the pharmacokinetics of trastuzumab using intravenous and subcutaneous (hind leg above the ankle) delivery to rats (Dahlberg et al., 2014). Compartments and rate constants are described in the text.

Insulin Absorption Models

Insulin was the first purified protein drug to become available for human use. Since its discovery in the early 1920s, multiple forms of insulin and insulin analogs with varying absorption and action profiles have become available. The therapeutic utility of insulin has been expanded by modifying the protein sequence (recombinant DNA technology) and altering the association and release properties of the formulation (using zinc, protamine, other excipients, and pH modifications) (Beals et al., 2013). Multiple factors have been shown to affect insulin absorption, including physiologic variables (such as temperature, exercise, and site and depth of injection) and product-dependent factors (type of insulin, concentration, and volume of injection), as reviewed by Hoffman and Ziv (1997), Nucci and Cobelli (2000), Charnam et al. (2001), and Wong et al. (2008).

An important intrinsic property of the insulin molecule that governs absorption is the ability for self-association. Most studies have assumed that only insulin monomers and dimers are small enough to be effectively absorbed, and the absorption delay was attributed to the time required for the hexamers to dissociate. However, experiments with nondissociating cobalt insulin have shown that it is absorbed in the form of hexamers, although the rate of absorption was slower as compared with the absorption of dimers (Brange et al., 1990). In addition, lymph collection experiments in sheep suggest that insulin is partly absorbed in the form of hexamers (Charman et al., 2001).

Simple compartmental models were used in early studies to describe insulin pharmacokinetics. For example, Kobayashi et al. (1983) used a single first-order rate constant to describe absorption after continuous subcutaneous infusion and subcutaneous bolus injection (Fig. 1A). Two transit compartments (injection site and interstitium) and two first-order rate constants (Fig. 1B) were used to characterize ultralente insulin pharmacokinetics. For example, Kobayashi et al. (1983) used a single first-order rate constant to describe absorption after continuous subcutaneous infusion and subcutaneous bolus injection (Fig. 1A).

Two transit compartments (injection site and interstitium) and two first-order rate constants (Fig. 1B) were used to characterize ultralente insulin pharmacokinetics. For example, Kobayashi et al. (1983) used a single first-order rate constant to describe absorption after continuous subcutaneous infusion and subcutaneous bolus injection (Fig. 1A). Two transit compartments (injection site and interstitium) and two first-order rate constants (Fig. 1B) were used to characterize ultralente insulin pharmacokinetics. For example, Kobayashi et al. (1983) used a single first-order rate constant to describe absorption after continuous subcutaneous infusion and subcutaneous bolus injection (Fig. 1A).

A combination of zero- and first-order kinetics was selected in a rat study (Fig. 1C) (Gopalakrishnan et al., 2005).

Other investigators proposed noncompartmental approaches for describing the absorption of different insulins. Berger and Rodbard (1989) used a two-parameter logistic equation to describe the absorption of four types of insulin (regular, neutral protamine Hagedorn [NPH], lente, and ultralente):

$$A\% (t) = 100 - \frac{100 \cdot t'}{(T_{50})^2 + t'^2}$$

(14)
where $A\%$ is the percentage of injected insulin remaining at the absorption site, $t$ is time after injection, $s$ is the steepness parameter, and $T_{50}$ is the time interval to permit 50% of the dose to be absorbed ($T_{50}$ was expressed as a linear function of the dose). Mosekilde et al. (1989) used three coupled partial differential equations to describe processes that were assumed to govern the absorption of soluble insulin: diffusion of free insulin, transformation between hexamers and dimers, binding of the dimers in the subcutaneous tissue, and absorption of insulin dimers. Degradation at the injection site was considered insignificant. Equations were solved numerically by dividing the subcutaneous layer into 20 rings, centered around the injection site.

The model was modified and extended to describe absorption of monomeric insulin analogs (Trajanoski et al., 1993) and glargine insulin (Tarin et al., 2005). Later, this model was incorporated into an interactive educational diabetes simulator to support clinical decisions (Lehmann et al., 2009). Although this model provides a more physiologic description of the processes at the injection site (as compared with conventional compartmental models), relatively complex mathematics and intensive computation requirement might preclude its application for future pharmacokinetic and pharmacodynamic analyses.

Wong et al. (2008) proposed a unifying model for six different insulins based on ordinary differential equations to simplify computation (Fig. 4). It was assumed that absorption of all types of insulin occurs through a common dimeric/monomeric compartment ($X_{dim}$). The entire dose of monomeric insulin was assumed to be injected into the dimeric/monomeric compartment. For regular insulin, the dose was distributed between the dimeric and hexameric compartments ($X_h$, and hexamers dissociated into dimers by a first-order process. The NPH, lente, and ultralente insulin models were similar to the model of regular insulin with an additional crystalline (C) state compartment. The NPH and lente insulin shared a common hexamic state with regular insulin, whereas ultralente insulin had a separate hexameric state with slower dissociation ($X_{h,ulente}$). For glargine insulin, separate precipitation ($P_{gla}$) and hexamic ($X_{h,gla}$) compartments were included, and the dissolution rate was dose dependent. The effect of the volume of injection was modeled by a rate of diffusive loss ($k_d$) (assuming a spherical depot). The delivery of insulin from the injection site into the systemic circulation occurred through a single transit compartment ($X_{tot}$, interstitium), and loss in this compartment was included ($k_{deg}$). Taken together, the model was able to describe the absorption kinetics for six different insulin types, including the concentration dependency for regular insulin and dose dependency for glargine insulin (Wong et al., 2008).

**Mechanistic Models for Subcutaneous Absorption of Monoclonal Antibodies**

Monoclonal antibodies are a distinct group of protein therapeutics with common structure and a high molecular weight that use antibody-specific mechanisms (i.e., binding to FcRn) to reduce degradation. Therefore, it can be hypothesized that different mAbs (and Fc-fusion proteins) may exhibit similar absorption patterns. Several studies have shown that binding to FcRn can assist the absorption of subcutaneous mAbs. Wang et al. (2008) reported that the subcutaneous bioavailability of 7E3 IgG1 was 3-fold higher in wild-type mice compared with FcRn-deficient animals. Antibody variants with increased binding affinity to FcRn at pH 6.0 and 7.4 showed increased and decreased bioavailability in mice, respectively (Deng et al., 2010, 2012). In contrast, no definitive effect of altering FcRn affinity on subcutaneous absorption was found for a series of IgG4 variants in cynomolgus monkeys (Datta-Mannan et al., 2012).

A series of studies by our group has systematically investigated multiple factors that influence the absorption of rituximab in rodents (Kagan et al., 2012, 2014; Kagan and Mager, 2013). Specifically, the following factors were evaluated: 1) the role of the anatomic location of the subcutaneous injection site (back, abdomen, and the dorsal side of the foot), 2) the volume of injection, 3) dose level, 4) binding to FcRn, 5) diffusion through the extracellular matrix, and 6) the rate of drug delivery. Rodents do not express human CD20 antigen, so rituximab pharmacokinetics are not affected by the antigen-antibody binding in these species, thus facilitating the analysis of the absorption kinetics. The anatomic site of injection had a major impact on the rate and the extent of subcutaneous absorption of rituximab in mice and rats, which is consistent with other studies (Beshyah et al., 1991; Macdougall et al., 1991; Jensen et al., 1994; Ter Braak et al., 1996; Kota et al., 2007). Interestingly, injection-site differences were not observed for golimumab in humans (Xu et al., 2010a). An inverse correlation was found between the dose level (1–40 mg/kg) and the subcutaneous bioavailability of rituximab in mice and rats (Kagan et al., 2012, 2014).

Modeling was used to distinguish between the two proposed mechanisms of dose-dependent absorption of rituximab: 1) FcRn-mediated protection from degradation at the injection site, and 2) FcRn-mediated transcytosis from the interstitial space to the blood. FcRn-mediated transport of IgG across cells has been demonstrated in vitro (Dickinson et al., 1999; Antohe et al., 2001). Only the model that incorporated binding of rituximab to a receptor (presumably FcRn) as a part of the absorption pathway was able to capture the observed dose-dependent bioavailability of rituximab (Kagan et al., 2012). The final absorption model included three competing processes at the absorption site: degradation of free rituximab ($k_{deg}$), absorption of free rituximab (presumably through the lymphatics, $k_{abs}$), and absorption of bound rituximab (presumably through FcRn-mediated transcytosis, $k_{abs}$) (Fig. 5A). The binding process was assumed to occur rapidly at the absorption site (ABS) and was characterized by the equilibrium dissociation constant ($K_{D}$), and the amount of binding sites was held constant ($R_{0}$). The following differential equations defined the absorption model:
where $C$ is the antibody concentration in the central compartment, $\text{ABS}_\text{free}$ is free rituximab at the absorption site, and $(\text{ABS}_\text{tot} - \text{ABS}_\text{free})$ is bound rituximab at the absorption site. Interestingly, the majority of model parameters were shared between the back and the abdomen injection sites (with the exception of the rate of absorption for free drug $k_a$), which was attributed to the differences in the subcutaneous space structure between two anatomic locations. A separate injection site compartment ($A_{\text{inj}}$) was included to account for a delay in the absorption observed in rats; this compartment was not required for describing the mouse data.

To further investigate the role of FcRn in the subcutaneous absorption of mAbs, rituximab was coadministered subcutaneously with a large amount of nonspecific IgG (500 mg/kg). The observed decrease in bioavailability was attributed to the saturation of FcRn, and the model provided a good prediction of the experimental results for different rituximab dose levels and injection sites based on parameters estimated for rituximab given alone (Kagan and Mager, 2013). For simplicity, the same affinity to FcRn was assumed for rituximab and IgG, and the amount of endogenous IgG at the absorption site was considered negligible. In addition, the model captured the pharmacokinetic profiles after coadministration of rituximab with subcutaneous hyaluronidase, which resulted in an increase in bioavailability, using parameters estimated for rituximab given alone, with the exception of the absorption rate constant for the free drug (Kagan and Mager, 2013). This result was in agreement with the mode of action of hyaluronidase (i.e., disruption of glycosaminoglycan matrix) (Bookbinder et al., 2006; Frost, 2007).

The model was applied for description of rituximab pharmacokinetics after intravenous and subcutaneous administration to mice. Several model parameters were shared between species, but others were scaled with body weight (see Interspecies Modeling of Subcutaneous Absorption for Protein Therapeutics) (Kagan et al., 2014). Simulations were used to help visualize the competition among the two absorption pathways and degradation kinetics (Kagan and Mager, 2013; Kagan et al., 2014). For rituximab given alone, receptor-mediated transport accounted for absorption of approximately 57, 22, and 9% of the dose for 1, 10, and 40 mg/kg dose levels (subcutaneous injection at the back to rats); this pathway was almost completely abrogated by coadministration with a large amount of nonspecific IgG (Kagan and Mager, 2013).

This mechanistic model was useful for evaluating the relative contribution of two pathways to subcutaneous bioavailability of rituximab (Kagan and Mager, 2013), and may be further applied for investigating the role of system- and drug-dependent parameters on the rate and extent of absorption of mAbs. For example, Fig. 6 shows that 10-fold difference in the binding affinity or the receptor expression level may have a significant effect on subcutaneous bioavailability of rituximab.

Dahlberg et al. (2014) used a combination of the first-order lymphatic uptake and Michaelis-Menten uptake by blood capillaries in their model of trastuzumab absorption in rats (Fig. 3B). A range of intravenous doses (0.02–2.0 mg/kg) and a single subcutaneous dose (2 mg/kg) were evaluated in non–lymph-cannulated and lymph-cannulated animals (see Mechanistic Dual-Pathway Subcutaneous Absorption Models). The estimated bioavailability of trastuzumab was 85.5%, which is similar to the extent of absorption reported for rituximab (70% at 1 mg/kg dose level in rats) (Kagan and Mager, 2013). The lymphatic pathway and the saturable uptake through blood capillaries accounted for 53.1 and 32.4% of the absorbed dose. The Michaelis-Menten kinetics were superior to the first-order kinetics in describing the absorption, and the estimated $K_m$ value suggested that this process was considerably saturated by the administered dose. The Michaelis-Menten kinetics as implemented in this study were similar to the receptor-mediated absorption mechanism of rituximab (as described before) (Kagan et al., 2012). A comparison of the Michaelis-Menten and receptor-binding models for describing the systemic disposition of drugs has been reported (Gibiansky et al., 2008; Yan et al., 2010).

The pharmacokinetics of anti-amyloid-β wild type and Fc-variant (I235A,H435A) mIgG2a was evaluated after a single intravenous and subcutaneous dose (5 mg/kg) in SCID mice (Deng et al., 2012). The bioavailability for the wild-type and Fc-variant antibody, lacking binding affinity to FcRn at pH 6.0 and 7.4, was 76.3 and 41.8%. A modification of the endosomal recycling model (previously used for describing the systemic disposition of mAbs by Hansen and Balthasar, 2013)–

\[
\frac{dA_{\text{inj}}}{dt} = -k_a \cdot A_{\text{inj}}
\]

\[
\frac{d\text{ABS}_\text{tot}}{dt} = k_a \cdot A_{\text{inj}} - (k_{\text{deg}} + k_a) \cdot \text{ABS}_\text{free} - k_2 \cdot (\text{ABS}_\text{tot} - \text{ABS}_\text{free})
\]

\[
\text{ABS}_\text{free} = 0.5 \cdot \left[ (\text{ABS}_\text{tot} - R^D_{\text{tot}} + K^D_{\text{tot}}) + \sqrt{(\text{ABS}_\text{tot} - R^D_{\text{tot}} + K^D_{\text{tot}})^2 + 4 \cdot K^D_{\text{tot}} \cdot \text{ABS}_\text{tot}} \right]
\]

\[
\frac{dC}{dt} = \frac{k_a \cdot \text{ABS}_\text{free}}{V_c} + k_2 \cdot \frac{(\text{ABS}_\text{tot} - \text{ABS}_\text{free})}{V_c} \pm \text{disposition}
\]
antibodies returned to the vascular space with a first-order process ($k_{ret}$, which was constrained to be equal to $k_{deg}$). The unbound fraction of IgG in the endosomal compartments was calculated (nonskin $f_{u1}$, skin $f_u2$). The competition between the exogenous test antibodies and endogenous murine IgG for FcRn binding was not considered due to the low endogenous IgG concentration in SCID mice. Fraction unbound ($f_u$) for Fc-variant antibody was set to 1. The model was able to simultaneously capture the data for both the wild-type and the Fc-variant mAbs (Deng et al., 2012).

Zhao et al. (2013) proposed a physiologic model of subcutaneous absorption of mAbs based on the structural tissue model developed by Garg and Balthasar (2007) for mAbs disposition. The majority of model parameters were fixed to previously published values. Lymphatic transit time and lymphatic elimination rate constant were estimated using the pharmacokinetic profiles of omalizumab in humans. Based on the sensitivity analysis, these investigators suggested that the lymphatic flow rate and lymphatic drug elimination constant were the most influential to bioavailability. However, it was mentioned that the extent of lymphatic elimination of mAbs remains unknown. Analysis of data for other mAbs administered by the subcutaneous route can be potentially used to further evaluate this model.

Population Modeling of Absorption and Covariate Analysis for Protein Therapeutics

Extravascular dosing commonly results in a greater variability as compared with intravenous administration. Quantification of the variability, in addition to the estimation of the structural parameters, is important in clinical settings for better understanding of the dosing requirements for an individual patient. At the same time, population variability may hamper identifiability of the structural parameters for complex mechanism-based models. Blood sampling during the absorption phase is often limited in clinical trials; and nonlinear disposition, frequently exhibited by protein therapeutics, may further obscure the absorption behavior. These factors may lead to over-simplification of the absorption component of the pharmacokinetic model. For example, between-subject variability is often omitted for the absorption rate and bioavailability parameters. Covariate analysis is rarely reported for absorption parameters; however, several studies have identified body size, age, and population characteristics as factors that may affect the rate of absorption (Agoram et al., 2007; Potocka et al., 2011; Naik et al., 2013).

Table 2 summarizes selected studies that used population modeling approach for therapeutic proteins. For example, the pharmacokinetics of pegylated erythropoietin, peginesatide (a 40-kDa PEG-peptide), filgrastim, and darbepoetin alfa were well described using a first-order absorption rate constant and a bioavailability term (Fig. 1A) (Jolling et al., 2005; Agoram et al., 2007; Krzyzanski et al., 2010; Naik et al., 2013). Chatelut et al. (1999) used a dual-pathway absorption model (with sequential zero-order and first-order absorption) (Fig. 1C) in population pharmacokinetic analysis of IFN-α2b after a single subcutaneous injection in patients with chronic hepatitis C infection. All absorption parameters were estimated with good precision, except for the absolute bioavailability (because of lacking intravenous data). Covariate analysis for absorption parameters was not performed, and the between-subject variability for these parameters was 33–40% (CV%). Saturable carrier-mediated membrane transport and precipitation with subsequent dissolution were discussed as potential reasons for complex absorption behavior. Other evaluated absorption models included zero-order or first-order only (with or without a lag time), and a combination of zero- and first-order processes occurring simultaneously. Models combining zero- and first-order absorption kinetics were reported for other proteins (Table 2).
A population model-based meta-analysis described the pharmacokinetics of recombinant human erythropoietin after intravenous and subcutaneous administration using data from 16 trials (Olsson-Gisleskog et al., 2007). Deconvolution analysis revealed complex absorption behavior, with a relatively dose-proportional first phase and more than dose-proportional second phase. The systemic disposition model included central and peripheral compartments and a combination of linear and saturable elimination. The best fit was obtained with a model that included two absorption mechanisms. The faster absorption phase was described as a sequential zero-order input into the depot compartment (with duration of 0.725 hour), followed by a first-order absorption into the central compartment. The slower absorption phase started after a delay (2.72 hours) and was described as a zero-order input directly into the central compartment (with duration of 37.8 hours). The fraction absorbed through the first pathway was 81 and 60% for dose levels smaller than or greater than 300 IU/kg, respectively. The absolute bioavailability was described by a hyperbolic function based on the dose level. The model successfully captured data from all studies, but the underlying mechanism for complex absorption remained unclear (Olsson-Gisleskog et al., 2007).

A recent review summarized a large number of human population pharmacokinetic studies for mAbs (given mostly by the intravenous route) (Dirks and Meibohm, 2010). Two-compartment model with linear elimination or a combination of linear and nonlinear clearances was usually used for mAbs. Body size was a commonly identified covariate for the volume of distribution and clearance. This review focuses on studies that performed population analysis for mAbs and Fc-fusion proteins given by the subcutaneous route (Table 3). First-order kinetics were selected for describing the absorption process after subcutaneous injection in all reviewed studies. Several studies reported evaluating zero-order kinetics or a combination of zero- and first-order processes. The absorption was generally slow, with the absorption rate constant ranging from 0.12 to 1.2 day⁻¹. The between-subject variability associated with the absorption rate constant was usually high or not estimated because of the limited data during the absorption phase. The absorption rate constant was found to decrease with age for canakinumab, denosumab, and AMG317, and the exponent on the centered covariate was from −0.319 to −0.72. A decrease of the lymph flow with age was suggested before as a mechanism of this behavior (Agoram et al., 2007).

In the majority of studies, covariate analysis for the absorption rate constant was not reported for mAbs. A high shrinkage for the estimate of $k_a$ precluded further evaluation of covariate effects on this parameter in several studies. Trials that evaluated intravenous administration estimated the absolute bioavailability, which ranged from 7 to 74%. For canakinumab, the bioavailability depended on the drug product (59–74%) (Chakraborty et al., 2012, 2013). The estimation of between-subject variability and the evaluation of covariates was generally not performed for the bioavailability parameter. Because of the lack of intravenous data, the apparent values for the clearance and the volume of distribution (CL/F and V/F) were estimated in several works, and body weight was frequently found to be a significant covariate. Therefore, dependence of the bioavailability on body weight and other covariates cannot be ruled out at this time.

**Interspecies Modeling of Subcutaneous Absorption for Protein Therapeutics**

The ability to predict pharmacokinetic and pharmacodynamic properties of drug candidates in humans based on preclinical data is essential for improving the success rate and predictability of drug development. In addition, development of approaches for sharing and scaling of parameters among species may provide an efficient way for integrative analysis of data from different species. Systemic disposition of protein therapeutics in humans could be predicted reasonably well using allometric relationships:

$$P = a \cdot BW^b$$

where $P$ is a parameter of interest, $BW$ is species body weight, and $a$ and $b$ are the coefficient and the exponent of the allometric equation. The value of the allometric exponents for clearance and the volume of distribution and the number of species required for the prediction has varied among studies (Mordenti et al., 1991; Mahmood, 2004, 2009; Ling et al., 2009; Wang and Pruensaritanont, 2010; Deng et al., 2011). Traditional allometry cannot be applied for drugs exhibiting a target-mediated disposition that results in dose-dependent pharmacokinetic parameters (Woo and Jusko, 2007; Kagan et al., 2010). We have previously combined target-mediated disposition models with allometric principles to simultaneously capture the pharmacokinetics of type I IFNs and exenatide in multiple species (Kagan et al., 2010; Chen et al., 2013).

The quantitative information on species difference in the absorption process is very limited. The first-order absorption rate constant for PEG-erythropoietin after subcutaneous injection was shown to scale with an allometric exponent of −0.147 (based on four species), and a species-specific bioavailability parameter was used (Jolling et al., 2005). The absorption of erythropoietin was described by a combination of zero- and first-order kinetics in three species (rats, monkeys, and humans) (Woo and Jusko, 2007). The relationship between the estimated parameters and species body weight was assessed using regression analysis, and the allometric exponent for the first-order absorption rate constant was calculated as −0.349. Dose-dependent rate and extent of absorption (frequently reported for protein therapeutics) can further complicate interspecies analysis. For example, separate values for the absorption rate constant or bioavailability are sometimes used for each dose level (Mager et al., 2003; Ramakrishnan et al., 2003; Gao and Jusko, 2012). Although this approach might be useful for capturing observed data, it provides little insight into the mechanism of absorption and cannot be used effectively for interspecies prediction.

Michaelis-Menten kinetics were used to successfully capture dose-dependent absorption of exenatide in multiple species, and species-specific parameters ($V_{\text{max}}$ and $K_m$) were required (Chen et al., 2013). The ability of the model to predict exenatide pharmacokinetics in humans after subcutaneous administration was evaluated by simulation using two approaches: 1) separate sets of $V_{\text{max}}$ and $K_m$ values from mice, rats, and monkeys; and 2) $V_{\text{max}}$ and $K_m$ for humans were calculated using the allometric approach from three preclinical species. Interestingly, human data could not be adequately predicted using monkey absorption parameters, but rat $V_{\text{max}}$ and $K_m$ provided results consistent with clinical data. The $V_{\text{max}}$ parameter was highly correlated with body weight (allometric exponent of 0.392, $R^2 = 0.996$). Although $K_m$ appeared less correlated with body weight (allometric exponent of 0.605, $R^2 = 0.602$), the combination of $V_{\text{max}}$ and $K_m$ values obtained from the regressions provided a good prediction of human data (Chen et al., 2013).

Recently, a mechanism-based model that combines the absorption of free and bound antibody with presystemic degradation was applied to describe the pharmacokinetics of rituximab in rats and mice (Kagan and Mager, 2013; Kagan et al., 2014). Subcutaneous absorption of rituximab was dose dependent in both species, but the absorption process was faster in mice, and the magnitude of the nonlinear absorption was less pronounced in mice as compared with rats. Similar structural model was able to capture the dose-dependent subcutaneous
<table>
<thead>
<tr>
<th>Drug</th>
<th>Species/Population</th>
<th>Absorption Model</th>
<th>Absorption Parameters</th>
<th>Between Subject</th>
<th>Covariates for Absorption</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Anakinra</td>
<td>Rats</td>
<td>Sequential zero- and first-order</td>
<td>Fraction of zero-order Duration of zero-order $k_a$</td>
<td>Yes for $k_a$</td>
<td>No</td>
<td>Liu et al., 2011</td>
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<td>Darbepoetin alfa</td>
<td>Anemic patients with chronic kidney disease</td>
<td>Zero-order input into the depot compartment followed by the first-order absorption Duration of input $k_a$</td>
<td>Yes for $k_a$</td>
<td>No linear function of the dose $k_a \propto (Age/50)^{-0.951}$</td>
<td>Doshi et al., 2010</td>
<td></td>
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<td>Darbepoetin alfa</td>
<td>Healthy humans</td>
<td>First-order</td>
<td>$k_a$</td>
<td>No</td>
<td>No</td>
<td>Agoram et al., 2007</td>
</tr>
<tr>
<td>Erythropoietin δ</td>
<td>Pediatric patients with chronic kidney disease</td>
<td>First-order</td>
<td>$F$</td>
<td>No</td>
<td>No</td>
<td>Knebel et al., 2008</td>
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<td>Erythropoietin</td>
<td>Healthy humans (meta-analysis)</td>
<td>(1) Zero-order input into the depot compartment followed by the first-order absorption Duration of zero-order $k_a$ $k_{a1}$ $k_{a2}$</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Olsson-Gisleskog et al., 2007</td>
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<td>Filgrastim</td>
<td>Healthy adults</td>
<td>Parallel zero- and first-order followed by first-order Duration of zero-order $k_a$</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Wiczling et al., 2009</td>
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<tr>
<td>Filgrastim</td>
<td>Healthy adults</td>
<td>First-order</td>
<td>$F$</td>
<td>No</td>
<td>No</td>
<td>Krzyzanski et al., 2010</td>
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<tr>
<td>Growth hormone</td>
<td>Healthy humans and dialysis patients</td>
<td>Michaelis-Menten</td>
<td>$V_{max}$ $K_m$ $k_a$ $T_{lag}$</td>
<td>No</td>
<td>$K_m$ population specific</td>
<td>Klitgaard et al., 2009</td>
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<tr>
<td>Insulin</td>
<td>Healthy humans</td>
<td>First-order with a transit compartment $F$</td>
<td>Yes $k_a$ $k_{al}$ $k_{at}$</td>
<td>No</td>
<td>$k_a \propto BMI$</td>
<td>Potocka et al., 2011</td>
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<td>Insulin</td>
<td>Rats</td>
<td>Parallel zero- and first-order</td>
<td>Fraction of zero-order Duration of zero-order $T_{lag}$ for zero-order $k_a$</td>
<td>No</td>
<td>No</td>
<td>Gopalakrishnan et al., 2005</td>
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<td>IFN-α2b</td>
<td>Human hepatitis C patients</td>
<td>Sequential zero- and first-order</td>
<td>$F$ - dose-dependent function Fraction of zero-order Duration of zero-order</td>
<td>Yes</td>
<td>No</td>
<td>Chatelut et al., 1999</td>
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<td>Leukemia inhibitory factor</td>
<td>Healthy postmenopausal women and infertile patients</td>
<td>Zero-order</td>
<td>Duration of zero-order $k_a$</td>
<td>No</td>
<td>No</td>
<td>Goggin et al., 2004</td>
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<td>Low molecular weight heparin</td>
<td>Healthy humans</td>
<td>Parallel zero- and first-order followed by first-order Duration of zero-order $T_{lag}$ for zero-order $k_a$</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Abe et al., 2013</td>
</tr>
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<td>Pegylated human erythropoietin</td>
<td>Rats, dogs, and monkeys</td>
<td>First-order</td>
<td>$F$ $k_a$ $k_{a1}$ $k_{a2}$ $T_{lag}$</td>
<td>Yes for $k_a$, $F$</td>
<td>$k_a \propto BW^{-0.149}$ $T_{lag} \propto BW^{-1.810}$ $F$ - species specific $k_a \propto BMI$, creatinine, ethnicity, dialysis status</td>
<td>Jolling et al., 2005</td>
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<td>Peginesatide</td>
<td>Chronic kidney disease patients</td>
<td>First-order</td>
<td>$T_{lag}$ $F$ $k_a$</td>
<td>Yes</td>
<td>$k_a \propto BMI$, creatinine, ethnicity, dialysis status</td>
<td>Naik et al., 2013</td>
</tr>
</tbody>
</table>

BMI, body mass index.

*The estimated parameters values allowed for two processes to occur simultaneously ($D_1 = 0.725$ h, $D_2 = 37.8$ h, $T_{lag} = 2.72$ h) (Olsson-Gisleskog et al., 2007).
absorption in both species (with the exception of an absorption delay for rats) (Fig. 5A). The binding affinity of rituximab to the receptor (presumably FcRn), the expression level of the receptor, and the rate constant for presystemic degradation were shared between species. The rate constants for absorption of the free and bound antibody were estimated separately for mice and rats, and decreased with increasing body weight. Interestingly, the relationships between the absorption rate constants and body weight was similar to the relationship between the distribution rate constants and body weight (Kagan et al., 2014).

**Conclusions and Future Perspective**

Subcutaneous absorption of proteins is regulated by a large number of factors, which can be divided into species, subject, and drug/formulation specific. Predicting subcutaneous absorption in humans is a difficult task, and the best preclinical model has not been identified (McDonald et al., 2010). Species differences in skin morphology and their effect on the rate and extent of subcutaneous absorption, and approaches for integrating this information into interspecies pharmacokinetic models require further investigation. Some physiologic processes that take part in the subcutaneous absorption may be scaled with body weight among species. Lindstedt and Schaeffer (2002) reported that the skin blood flow can be described using an allometric exponent of 0.741 using data for mouse, rat, dog, and human. Interestingly, the lymph flow rate in the thoracic duct can be scaled with an allometric exponent of 0.723 using data for mouse, rat, dog, and human.

**TABLE 3**

Selected studies that evaluated subcutaneous absorption of monoclonal antibodies and Fc-fusion proteins using population analysis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Population</th>
<th>Population Mean F, %</th>
<th>Population Mean ( k_a ), Day 1</th>
<th>Between Subject Variability for ( k_a ), CV%</th>
<th>Covariates for ( k_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adalimumab</td>
<td>Rheumatoid arthritis</td>
<td>58</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AMG317, fully human IgG2</td>
<td>Healthy humans</td>
<td>24.3</td>
<td>0.185</td>
<td>—</td>
<td>Age (centered at 40 yr) = —0.72b</td>
</tr>
<tr>
<td>Canakinumab</td>
<td>Human healthy and patients</td>
<td>63.3 or 70.0a</td>
<td>0.299 or 0.269a</td>
<td>64</td>
<td>Age (centered at 34 yr) = —0.555b</td>
</tr>
<tr>
<td>Canakinumab</td>
<td>Gouty arthritis</td>
<td>58.8–73.5a</td>
<td>0.193–0.296a</td>
<td>50.8</td>
<td>Age (centered at 52 yr) = —0.319b</td>
</tr>
<tr>
<td>Denosumab</td>
<td>Postmenopausal women with osteopenia or osteoporosis</td>
<td>63.8</td>
<td>0.212</td>
<td>63</td>
<td>Age (centered at 64 yr) = —0.577b</td>
</tr>
<tr>
<td>Denosumab</td>
<td>Bone metastasis</td>
<td>61.2</td>
<td>0.257</td>
<td>51.5</td>
<td>Age (centered at 54 yr) = —0.509b</td>
</tr>
<tr>
<td>Efalizumab</td>
<td>Psoriasis</td>
<td>56.4</td>
<td>0.242</td>
<td>—</td>
<td>Ng et al., 2005</td>
</tr>
<tr>
<td>Efalizumab</td>
<td>Psoriasis</td>
<td>—c</td>
<td>0.191</td>
<td>0 fixed</td>
<td>Sun et al., 2005</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Juvenile rheumatoid arthritis</td>
<td>58c</td>
<td>1.2</td>
<td>215</td>
<td>Yum et al., 2005</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Rheumatoid arthritis</td>
<td>—c</td>
<td>0.797</td>
<td>24.3 (73.1b)</td>
<td>Lee et al., 2003</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Psoriasis</td>
<td>—c</td>
<td>0.754</td>
<td>—</td>
<td>Nestorov et al., 2004</td>
</tr>
<tr>
<td>Fc-osteo protegerin</td>
<td>Healthy postmenopausal women</td>
<td>7.19</td>
<td>0.314</td>
<td>—</td>
<td>Zerhut et al., 2008</td>
</tr>
<tr>
<td>Golimumab</td>
<td>Psoriatic arthritis</td>
<td>—c</td>
<td>0.908</td>
<td>—</td>
<td>Xu et al., 2009</td>
</tr>
<tr>
<td>Golimumab</td>
<td>Rheumatoid arthritis</td>
<td>—c</td>
<td>0.125</td>
<td>—</td>
<td>Hu et al., 2011</td>
</tr>
<tr>
<td>Golimumab</td>
<td>Ankylosing spondylitis</td>
<td>—c</td>
<td>1.010</td>
<td>78.6</td>
<td>Xu et al., 2010b</td>
</tr>
<tr>
<td>IgG subcutaneous</td>
<td>Primary immunodeficiency</td>
<td>66.0</td>
<td>0.439</td>
<td>—</td>
<td>Landersdorfer et al., 2013</td>
</tr>
<tr>
<td>MTRX1011A, humanized</td>
<td>Rheumatoid arthritis</td>
<td>52.3 (IV 18.9 CV%)</td>
<td>0.212</td>
<td>68.7</td>
<td>Zheng et al., 2011</td>
</tr>
<tr>
<td>IgG1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omalizumab</td>
<td>Asthma</td>
<td>62c</td>
<td>0.480</td>
<td>39.9</td>
<td>Hayashi et al., 2007</td>
</tr>
<tr>
<td>Omalizumab</td>
<td>Asthma</td>
<td>—c</td>
<td>0.458</td>
<td>141</td>
<td>Lowe et al., 2009</td>
</tr>
<tr>
<td>Romiplostim</td>
<td>Healthy subjects</td>
<td>49.9</td>
<td>0.610</td>
<td>—</td>
<td>Wang et al., 2010</td>
</tr>
<tr>
<td>Sirukumab</td>
<td>Healthy subjects</td>
<td>—c</td>
<td>0.77</td>
<td>60.0</td>
<td>Zhang et al., 2013</td>
</tr>
<tr>
<td>TNFR-Fc fusion protein</td>
<td>Ankylosing spondylitis</td>
<td>—c</td>
<td>1.452 (( T_{lag} = 1 ) h)</td>
<td>55.6 (81.8 for ( T_{lag} ))</td>
<td>Fang et al., 2010</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>Psoriasis</td>
<td>—c</td>
<td>0.354</td>
<td>0 fixed</td>
<td>Zhi et al., 2009</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>Psoriatic arthritis</td>
<td>—c</td>
<td>0.427</td>
<td>82.4</td>
<td>Zhi et al., 2010</td>
</tr>
</tbody>
</table>

F, systemic bioavailability; IV, inter-individual variability; \( k_a \), first-order absorption rate constant; TNF, tumor necrosis factor.

aDepends on the drug product.

bExponent for body weight (power model).

cBioavailability was not modeled as a separate parameter; volume of distribution (\( V_d/F \)) and clearance (\( CL/F \)) were estimated. If the bioavailability value was reported, it was taken from previous studies.

cCV% for interoccasion variability.
space). The absorption of nonglycosylated granulocyte colony-stimulating factor (filgrastim) was faster and resulted in a greater AUC and Cmax (by 18 and 20%) as compared with glycosylated granulocyte colony-stimulating factor (lenograstim) after subcutaneous injection of 5 μg/kg in normal subjects (no difference between the elimination half-lives was observed) (Watts et al., 1997). Subcutaneous absorption of erythropoietin α in pediatric patients was 23% faster and resulted in a 43% lower bioavailability as compared with erythropoietin δ (erythropoietin δ is produced in a human cell line and has a different carbohydrate composition from Chinese hamster ovary [CHO] cell-derived erythropoietin α) (Knebel et al., 2008). Lower bioavailability was reported for mAbs with a higher pI values (more than ~9.0) in minipigs and humans (Zheng et al., 2012). Formulation related factors for subcutaneous injected biotherapeutics have been reviewed recently (Kinnunen and Mrsyny, 2014). However, quantitative characterization of the effects of formulation excipients and absorption modifiers on the rate and extent of absorption of therapeutic proteins should be investigated in future studies.

For insulin and mAbs, binding at the absorption site was proposed as a mechanism that modulates the systemic absorption (Mosekilde et al., 1989; Wang et al., 2008; Kagan et al., 2012); more information is needed regarding the effect of endogenous proteins, soluble receptors, and antidrug antibodies in the interstitial space on absorption of exogenous proteins. Presystemic degradation processes for therapeutic proteins are poorly understood, and in vitro incubation in subcutaneous tissue homogenates, lymph (or lymph nodes), and blood can be used for evaluating drug stability and informing the modeling process (Wang et al., 2012; Zou et al., 2013). However, in vitro studies might not fully represent the complexity of the in vivo conditions. Human growth hormone was stable for 6 hours in the central lymph of sheep, but there was a significant difference in the drug recovery between the peripheral and central lymph-cannulated groups (62 versus 8.6%) (Charman et al., 2000). Measurement of the disappearance rate of the injected protein from the subcutaneous site (in addition to plasma concentrations) can help to identify rate-limiting steps in the absorption process. A linear relationship was found between the half-life at the injection site and the molecular weight of four fluorescently labeled proteins (23 – 149 kDa) using whole-body imaging technique (Wu et al., 2012). The effect of various pathologic states on subcutaneous absorption has not been sufficiently characterized (e.g., obesity). Increased adiposity was associated with a reduced subcutaneous blood flow and a slower absorption of insulin in humans (Vora et al., 1992). Wang et al. (2012) reported that PEG40-erythropoietin exhibited approximately 2-fold lower exposure in fat rats as compared with normal rats after body-weight normalized subcutaneous dose administration.

In conclusion, this review summarized current experience with modeling of the pharmacokinetics of therapeutic proteins administered by the subcutaneous route. Despite continuing research in this area, our understanding of the quantitative aspects of subcutaneous absorption is still limited. It is anticipated that further development of mechanism-based modeling will play a central role in resolving complexities associated with absorption of these drugs.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Kagan.

References


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