6

Pharmacokinetics and Pharmacodynamics of Therapeutic Peptides and Proteins

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INTRODUCTION

The rational use of drugs and the design of effective dosage regimens are facilitated by the appreciation of the central paradigm of clinical pharmacology that there is a defined relationship between the administered dose of a drug, the resulting drug concentrations in various body fluids and tissues, and the intensity of pharmacologic effects caused by these concentrations (Meibohm and Derendorf 1997). This dose-exposure-response relationship and thus the dose of a drug required to achieve a certain effect are determined by the drug's pharmacokinetic and pharmacodynamic properties (Fig. 6.1).

Pharmacokinetics describes the time course of the concentration of a drug in a body fluid, preferably plasma or blood, that results from the administration of a certain dosage regimen. It comprises all processes affecting drug absorption, distribution, metabolism, and excretion. Simplified, pharmacokinetics characterizes "*what the body does to the drug*." In contrast, pharmacodynamics characterizes the intensity of a drug effect or toxicity resulting from certain drug concentrations in a body fluid, usually at the assumed site of drug action. It can be simplified to *what the drug does to the body* (Fig. 6.2) (Holford and Sheiner 1982; Derendorf and Meibohm 1999).

The understanding of the dose-concentration-effect relationship is crucial to any drug—including peptides and proteins—as it lays the foundation for dosing regimen design and rational clinical application. General pharmacokinetic and pharmacodynamic principles are to a large extent equally applicable to protein and peptide drugs as they are to traditional small molecule-based therapeutics. Deviations from some of these principles and additional challenges with regard to the characterization of the pharmacokinetics and pharmacodynamics of therapeutic peptides and proteins, however, arise from some of their specific properties:

(a) Their definition by the production process in a living organism rather than a chemically exactly defined structure and purity as it is the case for small-molecule drugs

(b) Their structural similarity to endogenous structural or functional proteins and nutrients

(c) Their intimate involvement in physiologic processes on the molecular level, often including regulatory feedback mechanisms

(d) The analytical challenges to identify and quantify them in the presence of a myriad of similar molecules

(e) Their large molecular weight and macromolecule character (for proteins)

This chapter will highlight some of the major pharmacokinetic properties and processes relevant for the majority of therapeutic peptides and proteins and will provide examples of well-characterized pharmacodynamic relationships for peptide and protein drugs. The clinical pharmacology of monoclonal antibodies, including special aspects in their pharmacokinetics and pharmacodynamics, will be discussed in further detail in Chap. 8. For a more general discussion on pharmacokinetic and pharmacodynamic principles, the reader is referred to several textbooks and articles that review the topic in extensive detail (see Further Reading).

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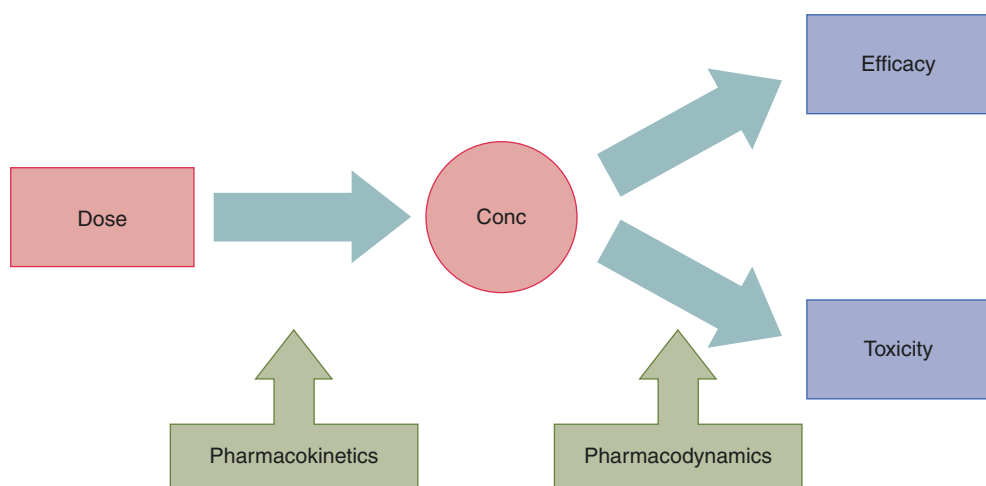
Figure 6.1 ■ The central paradigm of clinical pharmacology: the dose-concentration-effect relationship

PHARMACOKINETICS OF THERAPEUTIC PROTEINS

The in vivo disposition of peptide and protein drugs may often be predicted to a large degree from their physiological function (Tang and Meibohm 2006). Peptides, for example, which frequently have hormone activity, usually have short elimination half-lives, which is desirable for a close regulation of their endogenous levels and thus function. Insulin, for example, shows dose-dependent elimination with a relatively short half-life of 26 and 52 min at 0.1 and 0.2 U/kg, respectively. Contrary to that, proteins that have transport tasks such as albumin or long-term immunity functions such as immunoglobulins have elimination half-lives of several days, which enables and ensures the continuous maintenance of physiologically necessary concentrations in the bloodstream (Meibohm and Derendorf 2004). This is, for example, reflected by the elimination half-life of antibody drugs such as the anti-epidermal growth factor receptor antibody cetuximab, an IgG1 chimeric antibody for which a half-life of approximately 7 days has been reported (Herbst and Langer 2002).

■ Absorption of Therapeutic Proteins
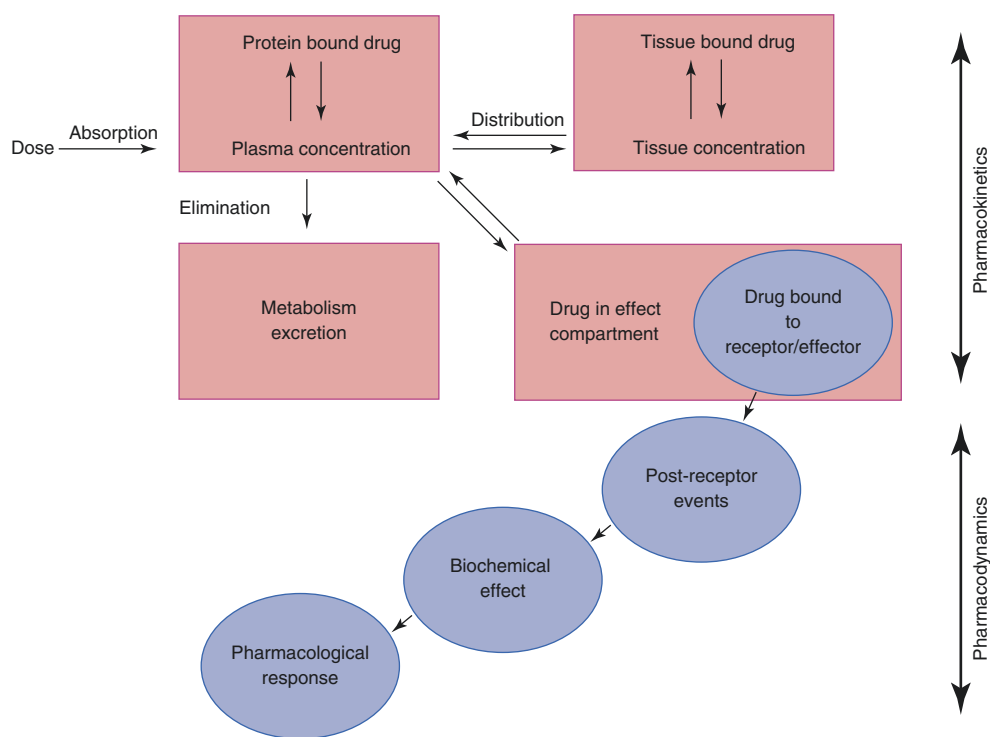#### *Enteral Administration*
Peptides and proteins, unlike conventional small-molecule drugs, are generally not therapeutically active upon oral administration (Fasano 1998; Mahato et al. 2003; Tang et al. 2004). The lack of systemic bioavailability is mainly caused by two factors: (1) high gastrointestinal enzyme activity and (2) low permeability through the gastrointestinal mucosa. In fact, the substantial peptidase and protease activity in the gastrointestinal tract makes it the most efficient body compartment for peptide and protein metabolism. Furthermore, the gastrointestinal mucosa presents a major absorption barrier for water-soluble macromolecules such as peptides and proteins (Tang et al. 2004). Thus, although various factors such as permeability, stability, and gastrointestinal transit time can affect the rate and extent of orally administered proteins, molecular size is generally considered the ultimate obstacle (Shen 2003).

Since oral administration is still a highly desirable route of delivery for protein drugs due to its convenience, cost-effectiveness, and painlessness, numerous strategies to overcome the obstacles associated with oral delivery of proteins have recently been an area of intensive research. Suggested approaches to increase the oral bioavailability of protein drugs include encapsulation into micro- or nanoparticles thereby protecting proteins from intestinal degradation (Lee 2002; Mahato et al. 2003; Shen 2003). Other strategies are chemical modifications such as amino acid backbone modifications and chemical conjugations to improve the resistance to degradation and permeability of the protein drug (Diao and Meibohm 2013). Coadministration of protease inhibitors has also been suggested for the inhibition of enzymatic degradation (Pauletti et al. 1997; Mahato et al. 2003). More details on approaches for oral delivery of therapeutic peptides and proteins are discussed in Chap. 5.

Parenteral Administration
Most peptide and protein drugs are currently formulated as parenteral formulations because of their poor oral bioavailability. Major routes of administration include intravenous (IV), subcutaneous (SC), and intramuscular (IM) administration. In addition, other non-oral administration pathways are utilized, including nasal, buccal, rectal, vaginal, transdermal, ocular, and pulmonary drug delivery (see Chap. 5).

Figure   6.2 ■ Physiological scheme of pharmacokinetic and pharmacodynamic processes

IV administration of peptides and proteins offers the advantage of circumventing presystemic degradation, thereby achieving the highest concentration in the biological system. Therapeutic proteins given by the IV route include, among many others, the tissue plasminogen activator (t-PA) analogues alteplase and tenecteplase, the recombinant human erythropoietin epoetin-α, and the granulocyte colony-stimulating factor filgrastim (Tang and Meibohm 2006).

IV administration as either a bolus dose or constant rate infusion, however, may not always provide the desired concentration-time profile depending on the biological activity of the product. In these cases, IM or SC injections may be more appropriate alternatives. For example, luteinizing hormone-releasing hormone (LH-RH) in bursts stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), whereas a continuous baseline level will suppress the release of these hormones (Handelsman and Swerdloff 1986). To avoid the high peaks from an IV administration of leuprorelin, an LH-RH agonist, a long-acting monthly depot injection of the drug is approved for the treatment of prostate cancer and endometriosis (Periti et al. 2002). A recent study comparing SC versus IV administration of epoetin-α in patients receiving hemodialysis reports that the SC route can maintain the hematocrit in a desired target range with a lower average weekly dose of epoetin-α compared to IV (Kaufman et al. 1998). In addition, SC injections have become increasingly popular as they allow self-administration by the patient, especially with the introduction of microneedles and pen devices, and thus not only circumvent the need to intravenous access, but also have increased patient acceptance and overall lower administration cost.

One of the potential limitations of SC and IM administration, however, are the presystemic degradation processes frequently associated with these administration routes, resulting in a reduced systemic bioavailability compared to IV administration. No correlation between the molecular weight of a therapeutic protein and its systemic bioavailability has so far been described in any species (Richter et al. 2012), and clinically observed bioavailability seems to be product-specific based on physicochemical properties and structure.

Bioavailability assessments for therapeutic proteins may be challenging if the protein exhibits the frequently encountered nonlinear pharmacokinetic behavior. Classic bioavailability assessments comparing systemic exposures quantified as area-under-the-concentration-time curve (AUC) resulting from extravascular versus IV administration assume linear pharmacokinetics, i.e., a drug clearance independent of concentration and the administration pathway. As this is not the case for many therapeutic proteins, especially those that undergo target-mediated drug disposition (see respective section in this

chapter), bioavailability assessments using the classic approach can result in substantial bias (Limothai and Meibohm 2011). Potential approaches suggested to minimize or overcome these effects include bioavailability assessments at doses at which the target- or receptor-mediated processes are saturated or to compare concentration-time profiles with similar shape and magnitude for extravascular and IV administration by modulating the input rate in the IV experiment.

The pharmacokinetically derived apparent absorption rate constant k_{app} for protein drugs administered via these administration routes is the combination of absorption into the systemic circulation and presystemic degradation at the absorption site, i.e., the sum of a true first-order absorption rate constant k_a and a first-order degradation rate constant. The true absorption rate constant k_a can then be calculated as

$$k_a = F \cdot k_{app}$$

where F is the systemic bioavailability compared to IV administration. A rapid apparent absorption, i.e., large k_{app}, can thus be the result of a slow true absorption and a fast presystemic degradation, i.e., a low systemic bioavailability (Colburn 1991).

Other potential factors that may limit the rate and/or extent of uptake of proteins after SC or IM administration include variable local blood flow, injection trauma, and limitations of uptake into the systemic circulation related to effective capillary pore size, diffusion, and convective transport.

Several therapeutic proteins including anakinra, etanercept, insulin, and pegfilgrastim, but also monoclonal antibodies such as adalimumab, omalizumab or alirocumab are administered as SC injections. Following a SC injection, therapeutic peptides and proteins may enter the systemic circulation either via blood capillaries or through lymphatic vessels (Porter and Charman 2000). There appears to be a defined relationship between the molecular weight of the protein and the proportion of the dose absorbed by the lymphatics (see Fig. 5.13) (Supersaxo et al. 1990). In general, peptides and proteins larger than 16 kDa are predominantly absorbed into the lymphatics, whereas those under 1 kDa are mostly absorbed into the blood circulation. While diffusion is the driving force for the uptake into blood capillaries, transport of larger proteins through the interstitial space into lymphatic vessels is mediated by convective transport with the interstitial fluid following the hydrostatic and osmotic pressure differences (see paragraphs on distribution). The fraction of insulin (5.2 kDa), for example, that has been described to undergo absorption through the lymphatic system is approximately 20% (see

Chap. 5 and 18), while this fraction is approaching 100% for monoclonal antibodies (150 kDa).

For monoclonal antibodies and fusion proteins with antibody Fc fragment, interaction with the neonatal Fc receptor (FcRn) has also been identified as a potential absorption process (Roopenian and Akilesh 2007). In this context, FcRn prevents the monoclonal antibody or fusion protein from undergoing lysosomal degradation (see Chap. 8 for details) and thereby increases systemic bioavailability, but may also facilitate transcellular transport from the absorption site into the vascular space. The contribution of this pathway to overall absorption, however, is limited.

Since lymph flow and interstitial convective transport are substantially slower than blood flow and diffusion processes, larger proteins taken up into lymphatic vessels usually show a delayed and prolonged absorption process after SC administration that can even become the rate-limiting step in their overall disposition. For monoclonal antibodies, for example, the time of the maximum concentration (t_{max}) was substantially delayed after SC administration, ranging from 1.7 to 13.5 days, with frequent values around 6–8 days (Zhao et al. 2013). A related model-based analysis suggests that lymphatic flow rate is the most influential factor for t_{max} of SC administered monoclonal antibodies.

Preferential uptake into lymphatic vessel after SC administration is of particular importance for those agents whose therapeutic targets are lymphoid cells (i.e., interferons and interleukins). Studies with recombinant human interferon α-2a (rhIFN α-2a) indicate that following SC administration, high concentrations of the recombinant protein are found in the lymphatic system, which drains into regional lymph nodes (Supersaxo et al. 1988). Due to this targeting effect, clinical studies show that palliative low-to-intermediate-dose SC recombinant interleukin-2 (rIL-2) in combination with rhIFN α-2a can be administered to patients in the ambulatory setting with efficacy and safety profiles comparable to the most aggressive IV rIL-2 protocol against metastatic renal cell cancer (Schomburg et al. 1993).

More recently, charge has also been described as an important factor in the SC absorption of proteins: While the positive and negative charges from collagen and hyaluronan in the extracellular matrix seem to be of similar magnitude, additional negative charges of proteoglycans may lead to a negative interstitial charge (Richter et al. 2012). This negative net charge and the associated ionic interactions with SC-administered proteins result in a slower transport for more positively rather than negatively charged proteins, as could be shown for several monoclonal antibodies (Mach et al. 2011).

■ Distribution of Therapeutic Proteins

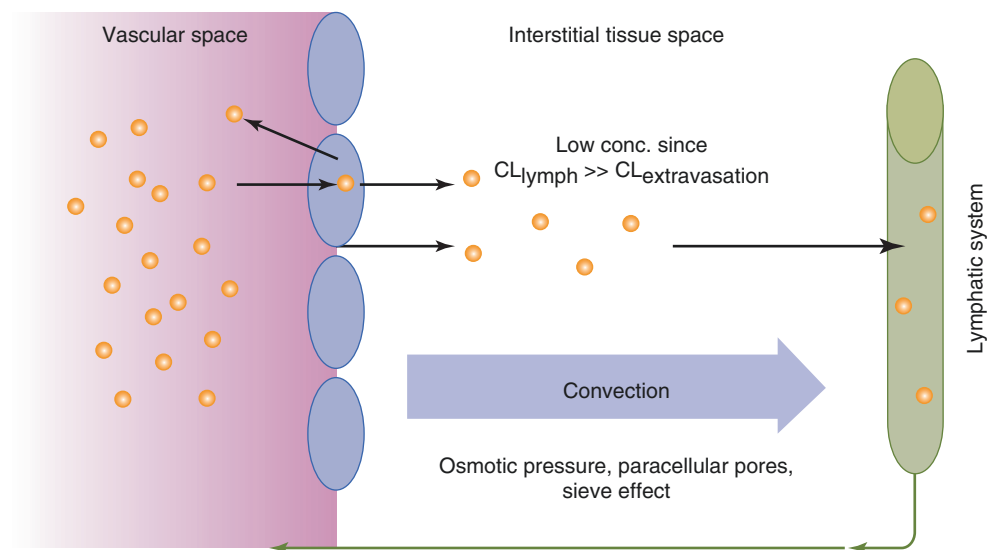*Distribution Mechanisms and Volumes*

The rate and extent of protein distribution is largely determined by the molecule size and molecular weight, physiochemical properties (e.g., charge, lipophilicity), binding to structural or transport proteins, and their dependency on active transport processes to cross biomembranes. Since most therapeutic proteins have high molecular weights and are thus large in size, their apparent volume of distribution is usually small and limited to the volume of the extracellular space due to their limited mobility secondary to impaired passage through biomembranes (Zito 1997). In addition, there is a mutual exclusion between therapeutic proteins and the structural molecules of the extracellular matrix. This fraction of the interstitial space that is not available for distribution is expressed as the excluded volume (Ve). It is dependent on the molecular weight and charge of the macromolecule and further limits extravascular distribution. For albumin (MW 66 kDa), the Ve has been reported as ~50% in muscle and skin (Ryman and Meibohm 2017). Active tissue uptake and binding to intra- and extravascular proteins, however, can substantially increase the apparent volume of distribution of protein drugs, as reflected by the relatively large volume of distribution of up to 2.8 L/kg for interferon β-1b (Chiang et al. 1993).

In contrast to small-molecule drugs, protein transport from the vascular space into the interstitial space of tissues is largely mediated by convection rather than diffusion, following the unidirectional fluid flux from the vascular space through paracellular pores into the interstitial tissue space (Fig. 6.3). The subsequent

removal from the interstitial space is accomplished by lymph drainage back into the systemic circulation (Flessner et al. 1997). This underlines the unique role the lymphatic system plays in the disposition of therapeutic proteins as already discussed in the section on absorption. The fact that the transfer clearance from the vascular to the interstitial space is smaller than the transfer clearance from the interstitial space to the lymphatic system results in lower protein concentrations in the interstitial space compared to the vascular space, thereby further limiting the apparent volume of distribution for therapeutic proteins. For endogenous and exogenous immunoglobulin G antibodies, for example, the tissue: blood concentration ratio is in the range of 0.1–0.5, i.e. antibody concentrations are substantially lower in the tissue interstitial fluid than in plasma (Ryman and Meibohm 2017). For brain tissue, the ratio is even in the range of 0.01 or lower, but may be higher in cases of compromised blood-brain barrier (Kingwell 2016).

Another, but much less prominent pathway for the movement of protein molecules from the vascular to the interstitial space is transcellular migration via endocytosis (Baxter et al. 1994; Reddy et al. 2006).

Besides the size-dependent sieving of macromolecules through the capillary walls, charge may also play an important role in the biodistribution of proteins. It has been suggested that the electrostatic attraction between positively charged proteins and negatively charged cell membranes might increase the rate and extent of tissue distribution. Most cell surfaces are negatively charged because of their abundance of glycosaminoglycans in the extracellular matrix.

Figure 6.3 ■ Distribution mechanisms of therapeutic proteins: convective extravasation rather than diffusion as major distribution process. $CL_{extravasation}$ transfer clearance from the vascular to the interstitial, CL_{lymph} transfer clearance from the interstitial space to the lymphatic system

After IV administration, peptides and proteins usually follow a biexponential plasma concentration-time profile that can best be described by a two-compartment pharmacokinetic model (Meibohm 2004). A biexponential concentration-time profile has, for example, been described for clenoliximab, a macaque-human chimeric monoclonal antibody specific to the CD4 molecule on the surface of T lymphocytes (Mould et al. 1999). Similarly, secukinumab, a human monoclonal antibody that binds and neutralizes interleukin 17A for the treatment of psoriasis, exhibited biphasic pharmacokinetics after IV administration (Bruin et al. 2017). The central compartment in this two-compartment model represents primarily the vascular space and the interstitial space of well-perfused organs with permeable capillary walls, including the liver and the kidneys. The peripheral compartment is more reflective of concentration-time profiles in the interstitial space of slowly equilibrating tissues.

The central compartment in which proteins initially distribute after IV administration has thus typically a volume of distribution equal or slightly larger than the plasma volume, i.e., 3–8 L. The total volume of distribution frequently comprises with 14–20 L not more than two to three times the initial volume of distribution (Colburn 1991; Dirks and Meibohm 2010). An example for such a distribution pattern is the t-PA analogue tenecteplase. Radiolabeled ^{125}I-tenecteplase was described to have an initial volume of distribution of 4.2–6.3 L and a total volume of distribution of 6.1–9.9 L with liver as the only organ that had a significant uptake of radioactivity. The authors concluded that the small volume of distribution suggests primarily intravascular distribution for tenecteplase, consistent with the drug's large molecular weight of 65 kDa (Tanswell et al. 2002).

Epoetin-α, for example, has a volume of distribution estimated to be close to the plasma volume at 0.056 L/kg after an IV administration to healthy volunteers (Ramakrishnan et al. 2004). Similarly, volume of distribution for darbepoetin-α has been reported as 0.062 L/kg after IV administration in patients undergoing dialysis (Allon et al. 2002), and distribution of thrombopoietin has also been reported to be limited to the plasma volume (~3 L) (Jin and Krzyzanski 2004).

It should be stressed that pharmacokinetic calculations of volume of distribution may be problematic for many therapeutic proteins (Tang et al. 2004; Straughn 2006). Noncompartmental determination of volume of distribution at steady state (V_{ss}) using statistical moment theory assumes first-order disposition processes with elimination occurring from the rapidly equilibrating or central compartment (Perrier and Mayersohn 1982; Straughn 1982; Veng-Pedersen and Gillespie 1984). These basic assumptions, however, are not fulfilled for numerous therapeutic proteins, as proteolysis and receptor-mediated elimination in peripheral tissues may constitute a substantial fraction of the overall elimination process. If therapeutic proteins are eliminated from slowly equilibrating tissues at a rate greater than their distribution process, substantial error in the volume of distribution assessment may occur. A recent simulation study could show that if substantial tissue elimination exists, a V_{ss} determined by noncompartmental methods will underestimate the "true" V_{ss} and that the magnitude of error tends to be larger the more extensively the protein is eliminated by tissue routes (Meibohm 2004; Straughn 2006; Tang and Meibohm 2006).

These challenges in characterizing the distribution of therapeutic proteins can only be overcome by determining actual protein concentrations in the tissue by biopsy or necropsy or via biodistribution studies with radiolabeled compound and/or imaging techniques.

Biodistribution studies are imperative for small organic synthetic drugs, since long residence times of the radioactive label in certain tissues may be an indication of tissue accumulation of potentially toxic metabolites. Because of the possible reutilization of amino acids from protein drugs in endogenous proteins, such a safety concern does not exist for therapeutic proteins. Therefore, biodistribution studies for protein drugs are usually only performed to assess drug targeting to specific tissues or to detect the major organs of elimination.

If a biodistribution study with radiolabeled protein is performed, either an external label such as ^{125}I can be chemically coupled to the protein if it contains a suitable amino acid such as tyrosine or lysine, or internal labeling can be used by growing the production cell line in the presence of amino acids labeled with ^{3}H, ^{14}C, ^{35}S, etc. The latter method, however, is not routinely used because of the prohibition of radioactive contamination of fermentation equipment (Meibohm and Derendorf 2004). Moreover, internally labeled proteins may be less desirable than iodinated proteins because of the potential reutilization of the radiolabeled amino acid fragments in the synthesis of endogenous proteins and cell structures. Irrespective of the labeling method, but more so for external labeling, the labeled product should have demonstrated physicochemical and biological properties identical to the unlabeled molecule (Bennett and McMartin 1978).

Protein Binding of Therapeutic Proteins

Another factor that can influence the distribution of therapeutic peptides and proteins is binding to endogenous protein structures. Physiologically active endog-

enous peptides and proteins frequently interact with specific binding proteins involved in their transport and regulation. Furthermore, interaction with binding proteins may enable or facilitate cellular uptake processes and thus affect the drug's pharmacodynamics.

It is a general pharmacokinetic principle, which is also applicable to proteins, that only the free, unbound fraction of a drug substance is accessible to distribution and elimination processes as well as interactions with its target structures at the site of action, for example, a receptor or ion channel. Thus, protein binding may affect the pharmacodynamics, but also disposition properties of therapeutic proteins. Specific binding proteins have been identified for numerous protein drugs, including recombinant human DNase for use as mucolytic in cystic fibrosis (Mohler et al. 1993), growth hormone (Toon 1996), and recombinant human vascular endothelial growth factor (rhVEGF) (Eppler et al. 2002).

Protein binding not only affects the unbound fraction of a protein drug and thus the fraction of a drug available to exert pharmacological activity, but many times it also either prolongs protein circulation time by acting as a storage depot or it enhances protein clearance. Recombinant cytokines, for example, may after IV administration encounter various cytokine-binding proteins including soluble cytokine receptors and anti-cytokine antibodies (Piscitelli et al. 1997). In either case, the binding protein may either prolong the cytokine circulation time by acting as a storage depot or it may enhance the cytokine clearance.

Growth hormone, as another example, has at least two binding proteins in plasma (Wills and Ferraiolo 1992). This protein binding substantially reduces growth hormone elimination with a tenfold smaller clearance of total compared to free growth hormone, but also decreases its activity via reduction of receptor interactions.

Ectodomain shedding is another source of binding proteins circulating in plasma where the extracellular domain of membrane-standing receptors is cleaved and released into the circulation (Hayashida et al. 2010). For therapeutic proteins targeting these receptors, the shed ectodomain constitutes a binding reservoir that by being in the vascular space is often more easily accessible than the intact membrane-standing receptor on target cells in the extravascular space. Thus, shed antigen can limit the disposition of a therapeutic protein and can inactivate a fraction of the administered therapeutic protein by preventing it from accessing its intended target (Ryman and Meibohm 2017). Different patients may have vastly different shed antigen concentrations and thus different effects, as shown for CD52, the target for alemtuzumab (Albitar et al. 2004).

Apart from these specific bindings, peptides and proteins may also be nonspecifically bound to plasma proteins. For example, metkephamid, a met-enkephalin analogue, was described to be 44–49% bound to albumin (Taki et al. 1998), and octreotide, a somatostatin analogue, is up to 65% bound to lipoproteins (Chanson et al. 1993).

Distribution Via Receptor-Mediated Uptake

Aside from physicochemical properties and protein binding of therapeutic proteins, site-specific receptor-mediated uptake can also substantially influence and contribute to the distribution of therapeutic proteins, as well as to elimination and pharmacodynamics (see section on "Target-Mediated Drug Disposition").

The generally low volume of distribution should not necessarily be interpreted as low tissue penetration. Receptor-mediated specific uptake into the target organ, as one mechanism, can result in therapeutically effective tissue concentrations despite a relatively small volume of distribution. Nartograstim, a recombinant derivative of the granulocyte colony-stimulating factor (G-CSF), for example, is characterized by a specific, dose-dependent, and saturable tissue uptake into the target organ bone marrow, presumably via receptor-mediated endocytosis (Kuwabara et al. 1995).

■ Elimination of Therapeutic Proteins

Therapeutic proteins are generally subject to the same catabolic pathways as endogenous or dietetic proteins. The end products of protein metabolism are thus amino acids that are reutilized in the endogenous amino acid pool for the de novo biosynthesis of structural or functional proteins in the human body (Meibohm 2004). Detailed investigations on the metabolism of proteins are relatively difficult because of the myriad of potential molecule fragments that may be formed, and are therefore generally not conducted. Non-metabolic elimination pathways such as renal or biliary excretion are negligible for most proteins. If biliary excretion occurs, however, it is generally followed by subsequent metabolic degradation of the compound in the gastrointestinal tract.

Proteolysis

In contrast to small-molecule drugs, metabolic degradation of peptides and therapeutic proteins by proteolysis can occur unspecifically nearly everywhere in the body. Due to this unspecific proteolysis of some proteins already in blood as well as potential active cellular uptake, the clearance of protein drugs can exceed cardiac output, i.e., >5 L/min for blood clearance and >3 L/min for plasma clearance (Meibohm 2004). The clearance of peptides or proteins in this context describes the irreversible removal of active substance

Molecular weight (kDa)	Elimination site	Predominant elimination mechanisms
<0.5	Blood, liver	Extracellular hydrolysis Passive lipoid diffusion
0.5–1	Liver	Carrier-mediated uptake Passive lipoid diffusion
1–60	Kidney	Glomerular filtration and subsequent degradation processes (see Fig. 6.4)
50–200	RES, endothelial cells (skin, muscle, gut), liver	Receptor-mediated endocytosis Pinocytosis
200–400	Immune system	Opsonization
>400	Phagocytic cells	Phagocytosis

Based on Meijer and Ziegler (1993) and Eigenmann et al. (2017)
Other determining factors are size, charge, lipophilicity, functional groups, sugar recognition, vulnerability for proteases, aggregation to particles, formation of complexes with opsonization factors, etc. As indicated, mechanisms may overlap. Endocytosis may occur at any molecular weight range; *RES* reticuloendothelial system

Table 6.1 ■ Molecular weight as major determinant of the elimination mechanisms of peptides and proteins

from the vascular space, which includes besides metabolism also cellular uptake. The metabolic rate for protein degradation generally increases with decreasing molecular weight from large to small proteins to peptides (Table 6.1), but is also dependent on other factors such as size, charge, lipophilicity, functional groups, and glycosylation pattern as well as secondary and tertiary structure.

Proteolytic enzymes such as proteases and peptidases are ubiquitous throughout the body. Sites capable of extensive peptide and protein metabolism are not only limited to the liver, kidneys, and gastrointestinal tissue, but also include blood and vascular endothelium as well as other organs and tissues. As proteases and peptidases are also located within cells, intracellular uptake is per se more an elimination rather than a distribution process (Tang and Meibohm 2006). While peptidases and proteases in the gastrointestinal tract and in lysosomes are relatively unspecific, soluble peptidases in the interstitial space and exopeptidases on the cell surface have a higher selectivity and determine the specific metabolism pattern of an organ. The proteolytic activity of subcutaneous and particularly lymphatic tissue, for example, results in a partial loss of activity of SC compared to IV administrated interferon-γ.

Gastrointestinal Protein Metabolism

As pointed out earlier, the gastrointestinal tract is a major site of protein metabolism with high proteolytic enzyme activity due to its primary function to digest dietary proteins. Thus, gastrointestinal metabolism is one of the major factors limiting systemic bioavailability of orally administered protein drugs. The metabolic activity of the gastrointestinal tract, however, is not limited to orally administered proteins. Parenterally administered peptides and proteins may also be metabolized in the intestinal mucosa following intestinal secretion. At least 20% of the degradation of endogenous albumin, for example, has been reported to take place in the gastrointestinal tract (Colburn 1991).
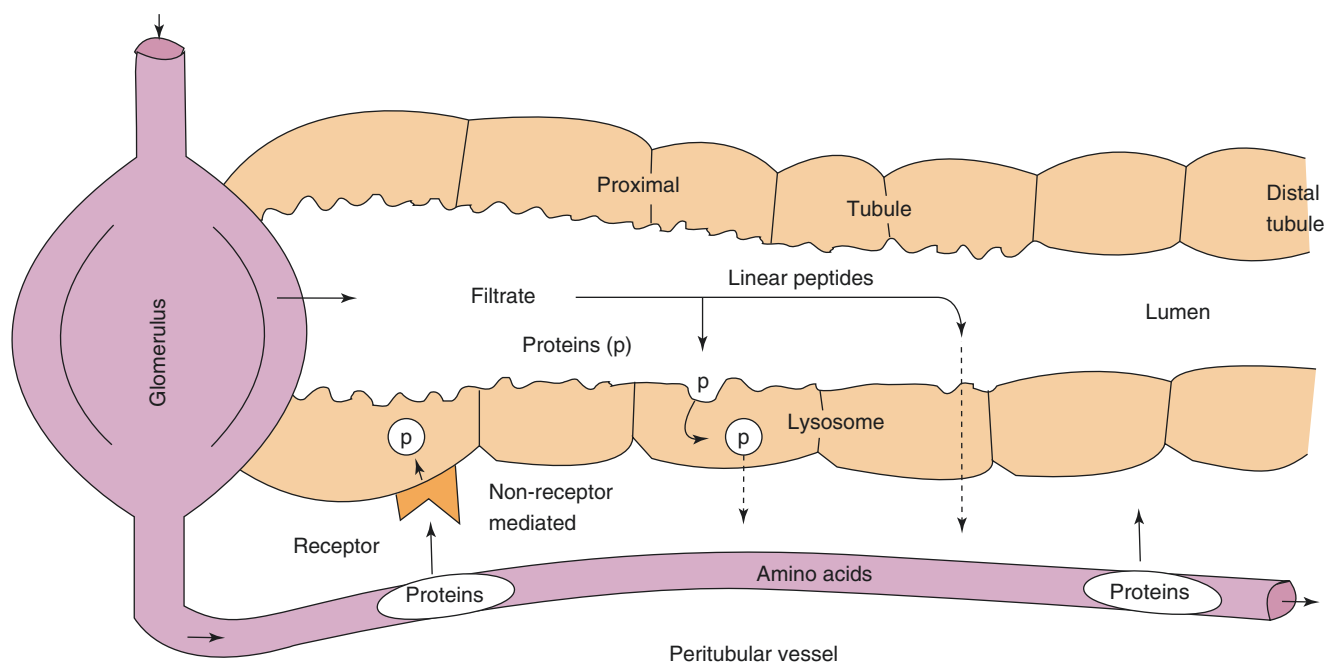
Renal Protein Metabolism

The kidneys are a major site of protein metabolism for smaller-sized proteins that undergo glomerular filtration. The size-selective cutoff for glomerular filtration is approximately 60 kDa, although the effective molecule radius based on molecular weight and conformation is probably the limiting factor (Edwards et al. 1999). Glomerular filtration is most efficient, however, for proteins smaller than 30 kDa (Kompella and Lee 1991). Peptides and small proteins (<5 kDa) are filtered very efficiently, and their glomerular filtration clearance approaches the glomerular filtration rate (GFR, ~120 mL/min in humans). For molecular weights exceeding 30 kDa, the filtration rate falls off sharply. In addition to size selectivity, charge selectivity has also been observed for glomerular filtration where anionic macromolecules pass through the capillary wall less readily than neutral macromolecules, which in turn pass through less readily than cationic macromolecules (Deen et al. 2001).

The importance of the kidneys as elimination organ could, for example, be shown for interleukin-2, macrophage colony-stimulating factor (M-CSF), and interferon-α (McMartin 1992; Wills and Ferraiolo 1992).

Renal metabolism of peptides and small proteins is mediated through three highly effective processes (Fig. 6.4). As a result, only minuscule amounts of intact protein are detectable in urine.

Figure 6.4 ■ Pathways of renal metabolism of peptides and proteins: glomerular filtration followed by either (1) intraluminal metabolism or (2) tubular reabsorption with intracellular lysosomal metabolism and (3) peritubular extraction with intracellular lysosomal metabolism (modified from Maack et al. 1985)

The first mechanism involves glomerular filtration of larger, complex peptides and proteins followed by reabsorption into endocytic vesicles in the proximal tubule and subsequent hydrolysis into small peptide fragments and amino acids (Maack et al. 1985). This mechanism of elimination has been described for IL-2 (Anderson and Sorenson 1994), IL-11 (Takagi et al. 1995), growth hormone (Johnson and Maack 1977), and insulin (Rabkin et al. 1984).

The second mechanism entails glomerular filtration followed by intraluminal metabolism, predominantly by exopeptidases in the luminal brush border membrane of the proximal tubule. The resulting peptide fragments and amino acids are reabsorbed into the systemic circulation. This route of disposition applies to small linear peptides such as glucagon and LH-RH (Carone and Peterson 1980; Carone et al. 1982). Recent studies implicate the proton-driven peptide transporters PEPT1 and especially PEPT2 as the main route of cellular uptake of small peptides and peptide-like drugs from the glomerular filtrate (Inui et al. 2000). These high-affinity transport proteins seem to exhibit selective uptake of di- and tripeptides, which implicates their role in renal amino acid homeostasis (Daniel and Herget 1997).

For both mechanisms, glomerular filtration is the dominant, rate-limiting step as subsequent degradation processes are not saturable under physiologic conditions (Maack et al. 1985; Colburn 1991). Under pathologic conditions or very high doses of the therapeutic protein, however, renal tubular reuptake processes may be overwhelmed, resulting in dose-dependent increases in urinary excretion of filtered proteins, as observed for the humanized monoclonal antibody Fab fragment (48 kDa) idarucizumab. The likely underlying mechanism is saturation of megalin and cubilin receptors on the apical membranes of renal tubular cells (Glund et al. 2018).

Due to this limitation of renal elimination, the renal contribution to the overall elimination of proteins is dependent on the proteolytic activity for these proteins in other body regions. If metabolic activity for these proteins is high in other body regions, there is only minor renal contribution to total clearance, and it becomes negligible in the presence of unspecific degradation throughout the body. If the metabolic activity is low in other tissues or if distribution to the extravascular space is limited, however, the renal contribution to total clearance may approach 100%.

The involvement of glomerular filtration in the renal metabolism of therapeutic proteins implies that the pharmacokinetics of therapeutic proteins below the molecular weight or hydrodynamic volume cutoff size for filtration will be affected by renal impairment. Indeed, it has been reported that the systemic exposure and elimination half-life increases with decreasing

glomerular filtration rate for recombinant human interleukin-10 (18 kDa), recombinant human growth hormone (22 kDa), and the recombinant human IL-1 receptor antagonist anakinra (17.3 kDa). Consistent with these theoretical considerations is also the observation that for monoclonal antibodies (150 kDa) such as rituximab, cetuximab, bevacizumab, trastuzumab and elotuzumab, no effect of renal impairment on their disposition has been reported (Meibohm and Zhou 2012; Berdeja et al. 2016).

The third mechanism of renal metabolism is peritubular extraction of peptides and proteins from postglomerular capillaries with subsequent intracellular metabolism. Experiments using radioiodinated growth hormone (^{125}I-rGH) have demonstrated that while reabsorption into endocytic vesicles at the proximal tubule is still the dominant route of disposition, a small percentage of the hormone may be extracted from the peritubular capillaries (Krogsgaard Thomsen et al. 1994). Peritubular transport of proteins and peptides from the basolateral membrane has also been shown for insulin (Nielsen et al. 1987).

Hepatic Protein Metabolism

Aside from renal and gastrointestinal metabolism, the liver may also play a major role in the metabolism of therapeutic proteins, especially for larger proteins. Exogenous as well as endogenous proteins undergo proteolytic degradation to dipeptides and amino acids that are reused for endogenous protein synthesis. Proteolysis usually starts with endopeptidases that attack in the middle part of the protein, and the resulting oligopeptides are then further degraded by exopeptidases. The rate of hepatic metabolism is largely dependent on the specific amino acid sequence of the protein (Meibohm 2004).

The major prerequisite for hepatic protein metabolism is the uptake of proteins in the different liver cell types. Small peptides may cross the hepatocyte membrane via simple passive diffusion if they have sufficient hydrophobicity. Peptides of this nature include cyclosporin (cyclic peptide) (Ziegler et al. 1988). Other cyclic and linear peptides of small size (<1.4 kDa) and hydrophobic nature (containing aromatic amino acids), such as cholecystokinin-8 (CCK-8; 8 amino acids), are taken up by the hepatocytes by carrier-mediated transport (Ziegler et al. 1988), in the case of CCK-8 by the organic anion-transporting polypeptide OATP-8 (SLCO1B3) (Ismair et al. 2001). After internalization into the cytosol, these peptides are usually metabolized by microsomal enzymes or cytosolic peptidases.

Uptake of larger peptides and proteins can either be facilitated through pinocytosis or by receptor-mediated endocytosis. Pinocytosis is an unspecific fluid-phase endocytosis, in which molecules are taken up into cells by forming invaginations of cell membrane around extracellular fluid, that are subsequently taken up as membrane vesicles.

Receptor-mediated endocytosis is a clathrin-mediated endocytosis process via relatively unspecific, promiscuous membrane receptors (McMahon and Boucrot 2011). In receptor-mediated endocytosis, circulating proteins are recognized by specific receptor proteins. The receptors are usually integral membrane glycoproteins with an exposed binding domain on the extracellular side of the cell membrane. After the binding of the circulating protein to the receptor, the complex is already present or moves in clathrin-coated pit regions, and the membrane invaginates and pinches off to form an endocytotic coated vesicle that contains the receptor and ligand (internalization). The vesicle coat consists of proteins (clathrin, adaptin, and others), which are then removed by an uncoating adenosine triphosphatase (ATPase). The vesicle parts, the receptor, and the ligands dissociate and are targeted to various intracellular locations. Some receptors, such as the low-density lipoprotein (LDL), asialoglycoprotein, and transferrin receptors, are known to undergo recycling. Since sometimes several hundred cycles are part of a single receptor's lifetime, the associated receptor-mediated endocytosis is of high capacity. Other receptors, such as the interferon receptor, undergo degradation. This degradation leads to a decrease in the concentration of receptors on the cell surface (receptor downregulation). Others, such as insulin receptors, for example, undergo both recycling and degradation (Kompella and Lee 1991).

For glycoproteins, receptor-mediated endocytosis through sugar-recognizing C-type lectin receptors is an efficient hepatic uptake mechanism if a critical number of exposed sugar groups (mannose, galactose, fucose, N-acetylglucosamine, N-acetylgalactosamine, or glucose) is exceeded (Meijer and Ziegler 1993). Important C-type lectin receptors in the liver are the asialoglycoprotein receptor on hepatocytes and the mannose and fucose receptors on Kupffer and liver endothelial cells (Smedsrod and Einarsson 1990; Bu et al. 1992). The high-mannose glycans in the first kringle domain of t-PA, for example, have been implicated in its hepatic clearance via these receptors (Cumming 1991).

The low-density lipoprotein receptor-related protein (LRP) is a member of the LDL receptor family responsible for endocytosis of several important lipoproteins, proteases, and protease-inhibitor complexes in the liver and other tissues (Strickland et al. 1995).

Uptake of proteins by liver cells is followed by transport to an intracellular compartment for metabolism. Proteins internalized into vesicles via an endocytotic mechanism undergo intracellular transport

towards the lysosomal compartment near the center of the cell. There, the endocytotic vehicles fuse with or mature into lysosomes, which are specialized acidic vesicles that contain a wide variety of hydrolases capable of degrading all biological macromolecules. Proteolysis is started by endopeptidases (mainly cathepsin D) that act on the middle part of the proteins. Oligopeptides—as the result of the first step—are further degraded by exopeptidases. The resulting amino acids and dipeptides reenter the metabolic pool of the cell. The hepatic metabolism of glycoproteins may occur more slowly than the naked protein because protecting oligosaccharide chains need to be removed first. Metabolized proteins and peptides in lysosomes from hepatocytes, hepatic sinusoidal cells, and Kupffer cells may be released into the blood. Degraded proteins in hepatocyte lysosomes can also be delivered to the bile canaliculus and excreted by exocytosis.

Besides intracellular degradation, a second intracellular pathway for proteins is the direct shuttle or transcytotic pathway (Kompella and Lee 1991). In this case, the endocytotic vesicle formed at the cell surface traverses the cell to the peribiliary space, where it fuses with the bile canalicular membrane, releasing its contents by exocytosis into bile. This pathway bypasses the lysosomal compartment completely. It has been described for polymeric immunoglobulin A but is not assumed to be a major elimination pathway for most protein drugs.

Target-Mediated Protein Metabolism

Therapeutic proteins frequently bind with high affinity to membrane-associated receptors on the cell surface if the receptors are the target structure to which the therapeutic protein is directed. This binding can lead to receptor-mediated uptake by endocytosis and subsequent intracellular lysosomal metabolism. The associated drug disposition behavior in which the binding to the pharmacodynamic target structure affects the pharmacokinetics of a drug compound is termed "target-mediated drug disposition" (Levy 1994).

For conventional small-molecule drugs, receptor binding is usually negligible compared to the total amount of drug in the body and rarely affects their pharmacokinetic profile. In contrast, a substantial fraction of a therapeutic protein can be bound to its pharmacologic target structure, for example, a receptor. Target-mediated drug disposition can affect distribution as well as elimination processes. Most notably, receptor-mediated protein metabolism is a frequently encountered elimination pathway for many therapeutic proteins (Meibohm 2004).

Receptor-mediated uptake and metabolism via interaction with these generally high-affinity, low-capacity binding sites is not limited to a specific organ or tissue type. Thus, any tissue including the therapeutically targeted cells that express receptors for the drug can contribute to the elimination of the therapeutic protein (Fig. 6.5) (Zhang and Meibohm 2012).

Since the number of protein drug receptors is limited, receptor-mediated protein metabolism can usually be saturated within therapeutic concentrations, or more specifically at relatively low molar ratios between the protein drug and the receptor (Mager 2006). As a consequence, the elimination clearance of these protein drugs is not constant but dose dependent and decreases with increasing dose. Thus, receptor-mediated elimination constitutes a major source for nonlinear pharmacokinetic behavior of numerous protein drugs, i.e., systemic exposure to the drug increases more than proportional with increasing dose (Tang et al. 2004).

Recombinant human macrophage colony-stimulating factor (M-CSF), for example, undergoes besides linear renal elimination a nonlinear elimination pathway that follows Michaelis-Menten kinetics and is linked to a receptor-mediated uptake into macrophages. At low concentrations, M-CSF follows linear pharmacokinetics, while at high concentrations nonrenal elimination pathways are saturated resulting in nonlinear pharmacokinetic behavior (Fig. 6.6) (Bauer et al. 1994).

Nonlinearity in pharmacokinetics resulting from target-mediated drug disposition has also been observed for several monoclonal antibody therapeutics, for instance for the anti-HER2 humanized monoclonal antibody trastuzumab. Trastuzumab is approved for the combination treatment of HER2 protein overexpressing metastatic breast cancer. With increasing dose level, the mean terminal half-life of trastuzumab increases and the clearance decreases, leading to overproportional increases in systemic exposure with increasing dose (Tokuda et al. 1999). Since trastuzumab is rapidly internalized via receptor-mediated endocytosis after binding to HER2, its target structure on the cell surface, saturation of this elimination pathway is the likely cause for the observed dose-dependent pharmacokinetics (Kobayashi et al. 2002).

Modulation of Protein Disposition by the FcRn Receptor

Immunoglobulin G (IgG)-based monoclonal antibodies and their derivatives as well as albumin conjugates constitute important classes of therapeutic proteins with many members currently being under development or in therapeutic use. Interaction with the neonatal Fc receptor (FcRn) constitutes a major component in the drug disposition of IgG molecules (Roopenian and Akilesh 2007). FcRn has been well-described in the transfer of passive humoral immunity from a mother to her fetus by transferring IgG
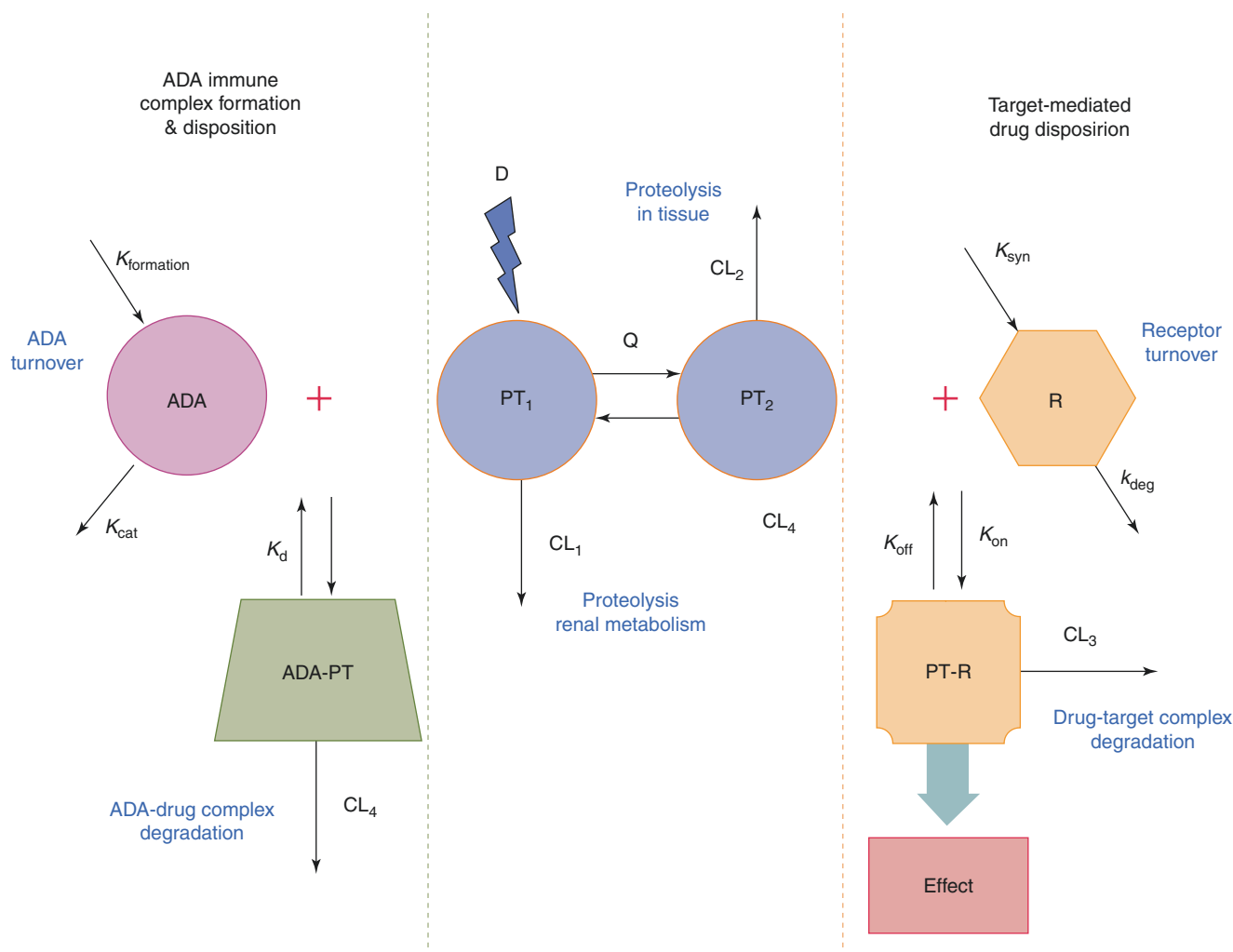
Figure 6.5 ■ Example of multiple clearance pathways affecting the pharmacokinetics of a typical therapeutic protein. Depicted is a two-compartment pharmacokinetic model with intravenous administration of a dose (D), concentrations of the therapeutic protein in the central (PT_1) and peripheral (PT_2) compartment, and interdepartmental clearance Q. The pharmacokinetic model includes two clearance pathways, one from the central compartment (CL_1) representative of, for example, renal metabolism or proteolytic degradation through the reticuloendothelial system and a second proteolytic degradation pathway from the peripheral compartment (CL_2) representative of, for example, proteolytic degradation through a receptor-mediated endocytosis pathway. Added to these two clearance pathways is on the right side a target-mediated disposition pathway that constitutes interaction of the therapeutic protein with its pharmacologic target receptor, which is in a homeostatic equilibrium of synthesis and degradation (synthesis rate k_{syn} and degradation rate constant k_{deg}). The dynamic equilibrium for the formation of the resulting therapeutic protein-receptor complex (PT-R) is determined through the association rate constant k_{on} and the dissociation rate constant k_{off}. The formation of PT-R does not only elicit the pharmacologic effect, but also triggers degradation of the complex. Thus, target binding and subsequent PT-R degradation constitute an additional clearance pathway for the therapeutic protein (CL_3). The left side of the graphic depicts the effect of an immune response to the therapeutic protein resulting in anti-drug antibody (ADA) formation. Again, the circulating concentration of the ADA is determined by a homeostatic equilibrium between its formation rate ($k_{formation}$) and a catabolic turnover process (rate constant k_{cat}). The ADA response results in the formation of immune complexes with the drug (ADA-PT). Dependent on the size and structure of the immune complexes, endogenous elimination pathways though the reticuloendothelial system may be triggered, most likely via Fcγ-mediated endocytosis. Thus, immune complex formation and subsequent degradation may constitute an additional clearance pathway (CL_4) for therapeutic proteins (from Chirmule et al. 2012)

across the placenta. More importantly, interaction with FcRn in a variety of cells, including endothelial cells and monocytes, macrophages, and other dendritic cells, protects IgG from lysosomal catabolism and thus constitutes a salvage pathway for IgG molecules that have been internalized in these cells types. This is facilitated by intercepting IgG in the endosomes and recycling it to the systemic circulation (Wang et al. 2008). The interaction with the FcRn receptor thereby prolongs the elimination half-life of
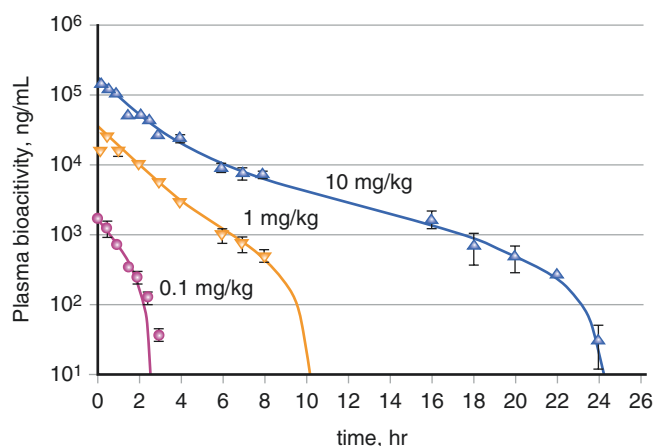
Figure 6.6 ■ Nonlinear pharmacokinetics of macrophage colony-stimulating factor (M-CSF), presented as measured (*triangles* and *circles*; mean ± SE) and modeled plasma bioactivity-time curves (*lines*) after intravenous injection of 0.1 mg/kg (*n* = 5), 1.0 mg/kg (*n* = 3), and 10 mg/kg (*n* = 8) in rats. Bioactivity is used as a substitute for concentration (from Bauer et al. 1994, with permission from American Society for Pharmacology and Experimental Therapeutics)
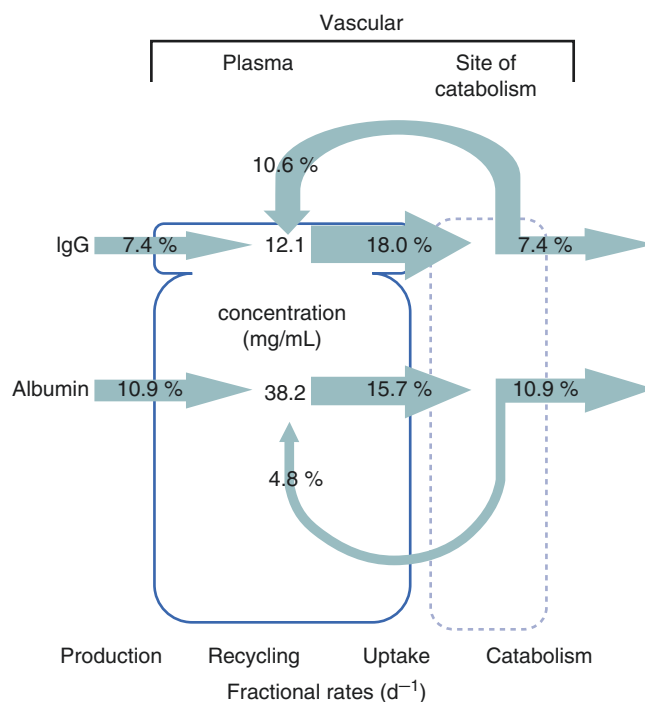

Figure 6.7 ■ Effect of FcRn-mediated recycling on IgG and albumin turnover in humans expressed as fractional rates. Shown are homeostatic plasma concentrations (12.1 and 38.2 mg/mL), fractional catabolic rates (7.4 and 10.9%/day), the FcRn-mediated fractional recycling rates (10.6 and 4.8%/day), and the fractional production rates (7.4 and 10.9%/day). The figure is to scale: areas for plasma amounts and arrow widths for rates (from Kim et al. 2007, with permission from Elsevier)

IgG, with a more pronounced effect the stronger the binding of the Fc fragment of the antibody is to the receptor: Based on the affinity of this binding interaction, human IgG1, IgG2, and IgG4 have a half-life in humans of 18–21 days, whereas the less strongly bound IgG3 has a half-life of only 7 days, and murine IgG in humans has a half-life of 1–2 days (Dirks and Meibohm 2010).

Similar to IgG, FcRn is also involved in the disposition of albumin molecules. The kinetics of IgG and albumin recycling are illustrated in Fig. 6.7. For IgG1, approximately 60% of the molecules taken up into lysosomes are recycled, for albumin 30%. As FcRn is responsible for the extended presence of IgG, albumin, and other Fc- or albumin-conjugated proteins in the systemic circulation, modulation of the interaction with FcRn allows to deliberately control the half-life of these molecules (Kim et al. 2007).

■ Immunogenicity and Protein Pharmacokinetics

The antigenic potential of therapeutic proteins may lead to antibody formation against the therapeutic protein during chronic therapy. This is especially of concern if animal-derived proteins are applied in human clinical studies, but also if human proteins are used in animal studies during preclinical drug development. Chapter 7 discusses in detail the phenomenon of immunogenicity and its consequences for the pharmacotherapy with therapeutic proteins.

The formation of anti-drug antibodies (ADA) against a therapeutic protein may not only modulate or even obliterate the biological activity of a protein drug,

but may also modify its pharmacokinetic profile. In addition, ADA-drug complex formation may lead to immune complex-mediated toxicity, particularly if the complexes get deposited in a specific organ or tissue. Glomerulonephritis has, for example, been observed after deposition of ADA-protein drug complexes in the renal glomeruli of Cynomolgus monkeys after intramuscular administration of rhINF-γ. Similar to other circulating immune complexes, ADA-protein drug complexes may trigger the regular endogenous elimination pathways for these complexes, which consist of uptake and lysosomal degradation by the reticuloendothelial system. This process has been primarily described for the liver and the spleen and seems to be mediated by Fcγ receptors.

The ADA formation may either lead to the formation of neutralizing or non-neutralizing ADA. Neutralizing ADA bind at or near the target-binding domain of the therapeutic protein and interfere with its ability to bind to its target receptor, thereby reducing its biologic activity. Non-neutralizing ADA bind to regions of the therapeutic protein that are more distant to the target-binding domain and do not interfere with its target binding. Independent on whether
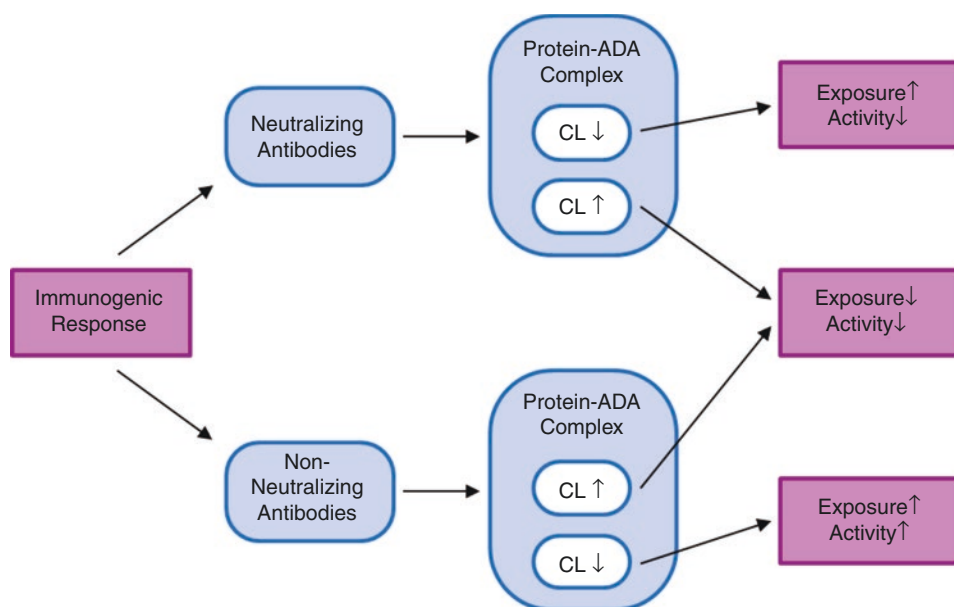
Figure 6.8 ■ Effect of anti-drug antibody (ADA) formation on the pharmacokinetics and pharmacodynamics of therapeutic proteins. *CL* clearance

ADA are neutralizing or non-neutralizing, they can both modulate the therapeutic protein's pharmacokinetics: Clearing ADA increase the clearance of the therapeutic protein, whereas sustaining ADA decrease the clearance of the therapeutic protein (Fig. 6.8). For clearing ADA, the immune complex formation triggers elimination via the reticuloendothelial system, which constitutes an additional elimination pathway for the protein (Fig. 6.5). This increase in clearance for the protein results in a decreased systemic exposure and reduced elimination half-life, which ultimately leads to reduced activity also for non-neutralizing ADA. A clearing effect of ADA is often observed for large therapeutic proteins such as monoclonal antibodies (Richter et al. 1999).

For sustaining ADA, the immune complex formation does not trigger the regular endogenous elimination processes, but serves as a storage depot for the protein, thereby reducing its clearance, increasing its systemic exposure, prolonging its half-life, and thereby increasing its activity in case of non-neutralizing ADA. This behavior has often been described for small therapeutic proteins where the immune complex formation, for example, prevents glomerular filtration and subsequent tubular metabolism. The elimination half-life of the therapeutic protein is then often increased to approach that of IgG (Chirmule et al. 2012).

Whether ADA-protein drug complex formation results in clearing or sustaining effects seems to be a function of its physicochemical and structural properties, including size, antibody class, ADA-antigen ratio, characteristics of the antigen, and location of the binding epitopes. For example, both an increased and decreased clearance is possible as ADA effect for the same protein, dependent on the dose level administered. At low doses, protein-antibody complexes delay clearance because their elimination is slower than the unbound protein. In contrast, at high doses, higher levels of protein-antibody complex result in the formation of larger aggregates, which are cleared more rapidly than the unbound protein.

The enhancement of the clearance of the cytokine interleukin-6 (IL-6) via administration of cocktails of three anti-IL-6 monoclonal antibodies was suggested as a therapeutic approach in cytokine-dependent diseases like multiple myeloma, B-cell lymphoma, and rheumatoid arthritis (Montero-Julian et al. 1995). The authors could show that, while the binding of one or two antibodies to the cytokine led to stabilization of the cytokine, simultaneous binding of three anti-IL-6 antibodies to three distinct epitopes induced rapid uptake of the complex by the liver and thus mediated a rapid elimination of IL-6 from the systemic circulation.

It should be emphasized that ADA formation is a polyclonal and usually relatively unspecific immune response to the therapeutic protein, with formation of different antibodies with variable binding affinities and epitope specificities, and that this ADA formation with its multiple-involved antibody species is different in different patients. Thus, reliable prediction of ADA formation and effects remains elusive at the current time (Chirmule et al. 2012).

The immunogenicity of therapeutic proteins is also dependent on the route of administration.

Extravascular injection is known to stimulate antibody formation more than IV application, which is most likely caused by the increased immunogenicity of protein aggregates and precipitates formed at the injection site. Further details on these aspects of immunogenicity are discussed in Chap. 7.

■ **Species Specificity and Allometric Scaling**

Peptides and proteins often exhibit distinct species specificity with regard to structure and activity. Peptides and proteins with identical physiological function may have different amino acid sequences in different species and may have no activity or be even immunogenic if used in a different species. The extent of glycosylation and/or sialylation of a protein molecule is another factor of species differences, e.g., for interferon-α or erythropoietin, which may not only alter its efficacy and immunogenicity (see Chap. 7) but also the drug's clearance.

Projecting human pharmacokinetic behavior for therapeutic proteins based on data in preclinical species is often performed using allometric approaches. Allometry is a methodology used to relate morphology and body function to the size of an organism. Allometric scaling is an empirical technique to predict body functions based on body size. Allometric scaling has found wide application in drug development, especially to predict pharmacokinetic parameters in humans based on the corresponding parameters in several animal species and the body size differences among these species and humans. Multiple allometric scaling approaches have been described with variable success rates, predominantly during the transition from preclinical to clinical drug development (Dedrick 1973; Boxenbaum 1982). In the

most frequently used approach, pharmacokinetic parameters between different species are related via body weight using a power function:

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

where P is the pharmacokinetic parameter scaled, W is the body weight in kg, a is the allometric coefficient, and b is the allometric exponent. a and b are specific constants for each parameter of a compound. General tendencies for the allometric exponent are 0.75 for clearances and flow rates, 1 for volumes of distribution, –0.25 for biologic rate constants, and 0.25 for half-lives. More recently, allometric approaches are being complemented by physiologically based pharmacokinetic modeling.

For most traditional small-molecule drugs, allometric scaling is often imprecise, especially if hepatic metabolism is a major elimination pathway and/or if there are interspecies differences in metabolism. For peptides and proteins, however, allometric scaling has frequently proven to be much more precise and reliable if their disposition is governed by relatively unspecific proteolytic degradation pathways. The reason is probably the similarity in handling peptides and proteins among different mammalian species (Wills and Ferraiolo 1992). Clearance and volume of distribution of numerous therapeutically used proteins like growth hormone or t-PA follow a well-defined, weight-dependent physiologic relationship between lab animals and humans. This allows relatively precise quantitative predictions for their pharmacokinetic behavior in humans based on preclinical findings (Mordenti et al. 1991).

Figure 6.9, for example, shows allometric plots for the clearance and volume of distribution of a
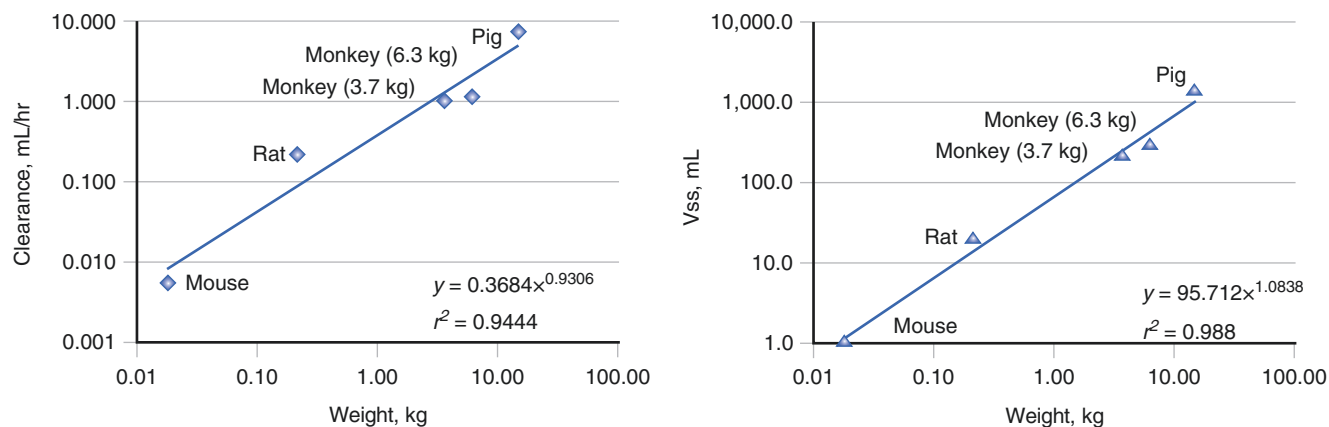
Figure 6.9 ■ Allometric plots of the pharmacokinetic parameter clearance and volume of distribution at steady state (V_{ss}) for the P-selectin antagonist rPSGL-Ig. Each data point within the plot represents an averaged value of the respective pharmacokinetic parameter in one of five species: mouse, rat, monkey (3.7 kg), monkey (6.3 kg), and pig, respectively. The *solid line* is the best fit with a power function to relate pharmacokinetic parameters to body weight (Khor et al. 2000; with permission from American Society for Pharmacology and Experimental Therapeutics)

P-selectin antagonist, P-selectin glycoprotein ligand-1, for the treatment of P-selectin-mediated diseases such as thrombosis, reperfusion injury, and deep vein thrombosis. The protein's human pharmacokinetic parameters could accurately be predicted using allometric power functions based on data from four species: mouse, rat, monkey, and pig (Khor et al. 2000).

Recent work on scaling the pharmacokinetics of monoclonal antibodies has suggested that allometric scaling from one nonhuman primate species, in this case the Cynomolgus monkey, using an allometric exponent of 0.85 might be superior to traditional allometric scaling approaches (Deng et al. 2011).

In any case, successful allometric scaling seems so far largely limited to unspecific protein elimination pathways. Once interactions with specific receptors are involved in drug disposition, for example, in receptor-mediated processes or target-mediated drug disposition, then allometric approaches oftentimes have large prediction error margins or even fail to scale drug disposition of therapeutic proteins across species due to differences in binding affinity and specificity, as well as expression and turnover kinetics of the involved receptors and targets in different species. In this situation, it becomes especially important to only consider for scaling preclinical pharmacokinetic data from "relevant" animal species for which the therapeutic protein shows cross-reactivity between animal and human receptors or targets. Dong et al. (2011) provided practical examples how unspecific and receptor-mediated elimination pathways for the same therapeutic protein can independently be scaled to improve human clearance predictions.

It needs to be emphasized that allometric scaling techniques are useful tools for predicting a dose that will assist in the planning of dose-ranging studies, including first-in-man studies, but are not a replacement for such studies. The advantage of including such dose prediction in the protocol design of dose-ranging studies is that a smaller number of doses need to be tested before finding the final dose level. Interspecies dose predictions simply narrow the range of doses in the initial pharmacological efficacy studies, the animal toxicology studies, and the human safety and efficacy studies. More recently, physiologically–based pharmacokinetic modeling has become more widely used to make more mechanistically based and accurate predictions of human pharmacokinetic behavior of therapeutic proteins (Diao and Meibohm 2015; Glassman and Balthasar 2016).

■ Chemical Modifications for Optimizing the Pharmacokinetics of Therapeutic Proteins

In recent years, approaches modifying the molecular structure of therapeutic proteins have repeatedly been applied to affect the immunogenicity, pharmacokinetics, and/or pharmacodynamics of protein drugs (Kontermann 2012). These approaches include the addition, deletion, or exchange of selected amino acids within the protein's sequence, synthesis of truncated proteins with a reduced amino acid sequence, glycosylation or deglycosylation, and covalent linkage to polymers (Veronese and Caliceti 2006). The latter approach has been used for several therapeutic proteins by linking them to monomethoxy polyethylene glycol (PEG) molecules of various chain lengths in a process called PEGylation (Caliceti and Veronese 2003).

The conjugation of high polymeric mass to protein drugs is generally aimed at preventing the protein being recognized by the immune system as well as reducing its elimination via glomerular filtration or proteolytic enzymes, thereby prolonging the oftentimes relatively short elimination half-life of endogenous proteins. Conjugation of protein drugs with PEG chains increases their molecular weight, but because of the attraction of water molecules by PEG even more their hydrodynamic volume, this in turn results in a reduced renal clearance and restricted volume of distribution. PEGylation can also shield antigenic determinants on the protein drug from detection by the immune system through steric hindrance (Walsh et al. 2003). Similarly, amino acid sequences sensitive towards proteolytic degradation may be shielded against protease attack. By adding a large, hydrophilic molecule to the protein, PEGylation can also increase drug solubility (Molineux 2003).

PEGylation has been used to improve the therapeutic properties of numerous therapeutic proteins including interferon-α, asparaginase, and filgrastim. More details on the general concept of PEGylation and its specific application for therapeutic proteins can be found in Chap. 27.

The therapeutic application of L-asparaginase in the treatment of acute lymphoblastic leukemia has been hampered by its strong immunogenicity with allergic reactions occurring in 33–75% of treated patients in various studies. The development of pegaspargase, a PEGylated form of L-asparaginase, is a successful example for overcoming this high rate of allergic reactions towards L-asparaginase using PEG conjugation techniques (Graham 2003). Pegaspargase is well-tolerated compared to L-asparaginase, with 3–10% of the treated patients experiencing clinical allergic reactions.

Pegfilgrastim is the PEGylated version of the granulocyte colony-stimulating factor filgrastim, which is administered for the management of chemotherapy-induced neutropenia. PEGylation minimizes filgrastim's renal clearance by glomerular filtration, thereby making neutrophil-mediated clearance
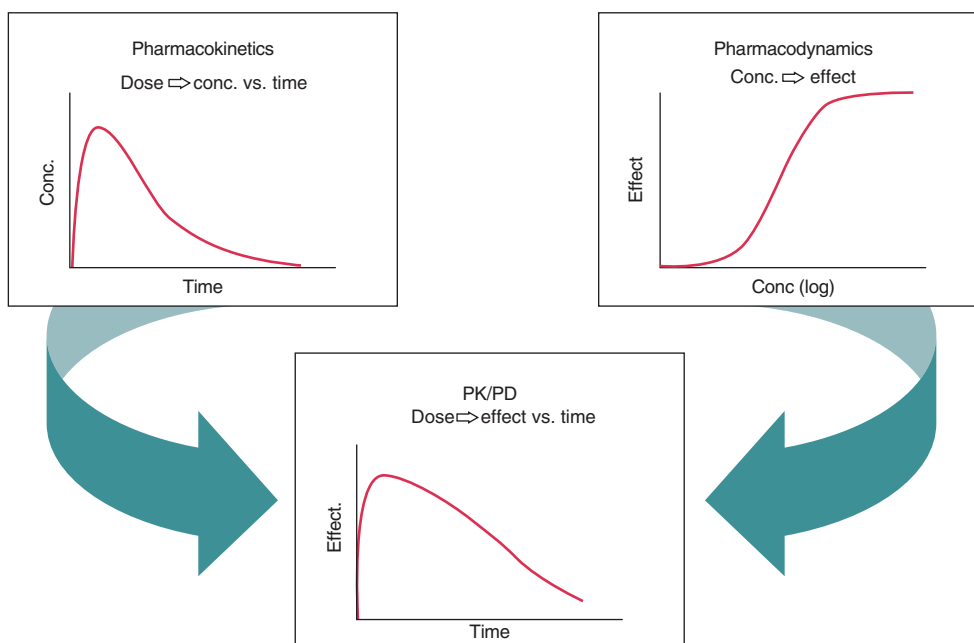
Figure 6.10 ■ General concept of PK/PD modeling. Pharmacokinetic-pharmacodynamic (PK/PD) modeling combines a pharmacokinetic model component that describes the time course of drug in plasma and a pharmacodynamic model component that relates the plasma concentration to the drug effect in order to describe the time course of the effect intensity resulting from the administration of a certain dosage regimen (from Derendorf and Meibohm 1999)

the predominant route of elimination. Thus, PEGylation of filgrastim results in so-called self-regulating pharmacokinetics since pegfilgrastim has a reduced clearance and thus prolonged half-life and more sustained duration of action in a neutropenic compared to a normal patient because only few mature neutrophils are available to mediate its elimination (Zamboni 2003).

The hematopoietic growth factor darbepoetin-α is an example of a chemically modified endogenous protein with altered glycosylation pattern. It is a glycosylation analogue of human erythropoietin, with two additional N-linked oligosaccharide chains (five in total) (Mould et al. 1999). The additional N-glycosylation sites were made available through substitution of five amino acid residues in the peptide backbone of erythropoietin, thereby increasing the molecular weight from 30 to 37 kDa. Darbepoetin-α has a substantially modified pharmacokinetic profile compared to erythropoietin, resulting in a threefold longer serum half-life that allows for reduced dosing frequency. More details on hematopoietic growth factors, including erythropoietin and darbepoetin-α, are provided in Chap. 24.

PHARMACODYNAMICS OF THERAPEUTIC PROTEINS

Therapeutic proteins are usually highly potent compounds with steep dose-effect curves as they are targeted therapies towards a specific, well-described pharmacologic structure or mechanism. Thus, a careful characterization of the concentration-effect relationship, i.e., the pharmacodynamics, is especially desirable for therapeutic proteins (Tabrizi and Roskos 2006). Combination of pharmacodynamics with pharmacokinetics by integrated pharmacokinetic-pharmacodynamic modeling (PK/PD modeling) adds an additional level of complexity that allows furthermore characterization of the dose-exposure-response relationship of a drug and a continuous description of the time course of effect intensity directly resulting from the administration of a certain dosage regimen (Fig. 6.10) (Meibohm and Derendorf 1997; Derendorf and Meibohm 1999).

PK/PD modeling is a technique that combines the two classical pharmacologic disciplines of pharmacokinetics and pharmacodynamics. It integrates a pharmacokinetic and a pharmacodynamic model component into one set of mathematical expressions that allows the description of the time course of effect intensity in response to administration of a drug dose. This so-called integrated PK/PD model allows deriving pharmacokinetic and pharmacodynamic model parameters that characterize the dose-concentration-effect relationship for a specific drug based on measured concentration and effect data. In addition, it allows simulation of the time course of effect intensity for dosage regimens of a drug beyond actually measured data, within the constraints of the validity of the model assumptions for the simulated condition. Addition of a statistical model component describing inter- and intraindividual

variation in model parameters allows expanding PK/PD models to describe time courses of effect intensity not only for individual subjects, but also for whole populations of subjects.

Integrated pharmacokinetic-pharmacodynamic (PK/PD) modeling approaches have widely been applied for the characterization of therapeutic proteins (Tabrizi and Roskos 2006). Embedded in a model-informed drug development paradigm, modeling and simulation based on integrated PK/PD does not only provide a comprehensive summary of the available data, but also enables to test competing hypotheses regarding processes altered by the drug, allows making predictions of drug effects under new conditions, and facilitates to estimate inaccessible system variables (Meibohm and Derendorf 1997; Mager et al. 2003).

Mechanism-based PK/PD modeling appreciating the physiological events involved in the elaboration of the observed effect has been promoted as superior modeling approach as compared to empirical modeling, especially because it does not only describe observations but also offers some insight into the underlying biological processes involved and thus provides flexibility in extrapolating the model to other clinical situations (Levy 1994; Derendorf and Meibohm 1999; Suryawanshi et al. 2010). Since the molecular mechanism of action of a therapeutic protein is generally well-understood, it is often straightforward to transform this available knowledge into a mechanism-based PK/PD modeling approach that appropriately characterizes the real physiological process leading to the drug's therapeutic effect.

The relationship between exposure and response may be either simple or complex, and thus obvious or hidden. However, if no simple relationship is obvious, it would be misleading to conclude a priori that no relationship exists at all rather than that it is not readily apparent (Levy 1986).

The application of PK/PD modeling is beneficial in all phases of preclinical and clinical drug development and has been endorsed by the pharmaceutical industry, academia, and regulatory agencies (Peck et al. 1994; Lesko et al. 2000; Sheiner and Steimer 2000; Meibohm and Derendorf 2002; Lesko 2007). Thus, PK/PD concepts and model-based drug development play a pivotal role especially in the drug development process for biologics, and their widespread application supports a scientifically driven, evidence-based, and focused product development for therapeutic proteins (Zhang et al. 2008; Mould and Meibohm 2016).

While a variety of PK/PD modeling approaches has been employed for biologics, we will in the following focus on five classes of approaches to illustrate the challenges and complexities, but also opportunities to characterize the pharmacodynamics of therapeutic proteins:

- Direct link PK/PD models
- Indirect link PK/PD models
- Indirect response PK/PD models
- Cell life span models
- Complex response models

It should not be unmentioned, however, that PK/PD models for therapeutic proteins are not only limited to continuous responses as shown in the following, but are also used for binary or graded responses. Binary responses are responses with only two outcome levels where a condition is either present or absent, e.g., dead versus alive. Graded or categorical responses have a set of predefined outcome levels, which may or may not be ordered, for example, the categories "mild," "moderate," and "severe" for a disease state. Lee et al. (2003), for example, used a logistic PK/PD modeling approach to link cumulative AUC of the anti-TNF-α protein etanercept with a binary response, the American College of Rheumatology response criterion of 20% improvement (ARC20) in patients with rheumatoid arthritis.
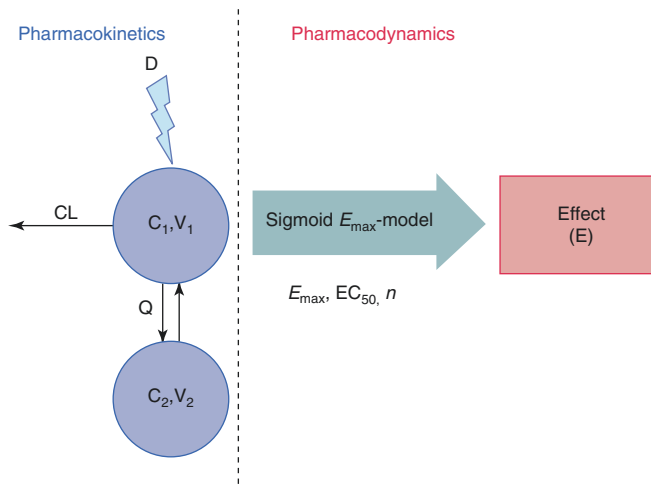
■ Direct Link PK/PD Models

The concentration of a therapeutic protein is usually only quantified in plasma, serum, or blood, while the magnitude of the observed response is determined by the concentration of the protein drug at its effect site, the site of action in the target tissue (Meibohm and Derendorf 1997). Effect site concentrations, however, are usually not accessible for measurement, and plasma, serum, or blood concentrations are usually used as their substitute. The relationship between the drug concentration in plasma and at the effect site may either be constant or undergo time-dependent changes. If equilibrium between both concentrations is rapidly achieved or the site of action is within plasma, serum, or blood, there is practically a constant relationship between both concentrations with no temporal delay between plasma and effect site. In this case, measured plasma concentrations can directly serve as input for a pharmacodynamic model (Fig. 6.11). The most frequently used direct link pharmacodynamic model is a sigmoid E_{max} model:

$$E = \frac{E_{max} \cdot C^n}{EC_{50}^n + C^n}$$

with E_{max} as maximum achievable effect, C as drug concentration in plasma, and EC_{50} the concentration of the drug that produces half of the maximum effect. The Hill coefficient n is an empirical shape factor that allows for an improved fit of the relationship to

the observed data. As represented by the equation for the sigmoid E_{max} model, a direct link model directly connects measured concentration to the observed effect without any temporal delay (Derendorf and Meibohm 1999).


Figure 6.11 ■ Schematic of a typical direct link PK/PD model. The PK model is a typical two-compartment model with a linear elimination clearance from the central compartment (*CL*) and a distributional clearance (*Q*). C_1 and C_2 are the concentrations in the central and peripheral compartments, and V_1 and V_2 are their respective volumes of distribution. The effect (*E*) is directly linked to the concentration in the central compartment C_1 via a sigmoid E_{max} model. The sigmoid E_{max} relationship is characterized by the pharmacodynamic parameters E_{max}, the maximum achievable effect, EC_{50}, the concentration of the drug that produced half of the maximum effect, and the Hill coefficient n as via the sigmoid E_{max} equation

A direct link model was, for example, used to relate the serum concentration of the antihuman immunoglobulin E (IgE) antibody CGP 51901 for the treatment of seasonal allergic rhinitis to the reduction of free IgE via an inhibitory E_{max} model (Fig. 6.12) (Racine-Poon et al. 1997). It should be noted that the peak and trough concentrations and effects are directly related and thus occur at the same times, respectively, without time delay. Similarly, a direct link model was used to relate the effect of recombinant interleukin-10 (IL-10) on the ex vivo release of the pro-inflammatory cytokines TNF-α and interleukin-1β in LPS-stimulated leukocytes (Radwanski et al. 1998). In the first case, the site of action and the sampling site for concentration measurements of the therapeutic protein were identical, i.e., in plasma, and so the direct link model was mechanistically well justified. In the second case, the effect was dependent on the IL-10 concentration on the cell surface of leukocytes where IL-10 interacts with its target receptor. Again sampling fluid and effect site were in instant equilibrium.

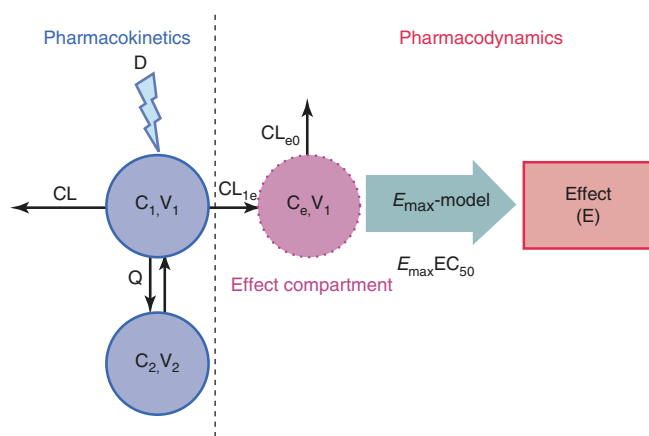### ■ Indirect Link PK/PD Models

The concentration-effect relationship of many protein drugs, however, cannot be described by direct link PK/PD models, but is characterized by a temporal dissociation between the time courses of plasma concentration and effect. In this case, plasma concentration maxima occur before effect maxima; effect intensity may increase despite decreasing plasma concentrations and may persist beyond the time when drug concentrations in plasma are no longer detectable. The relationship between measured concentration and observed effect follows a counterclockwise hysteresis loop. This phenomenon can either be caused by an indirect response
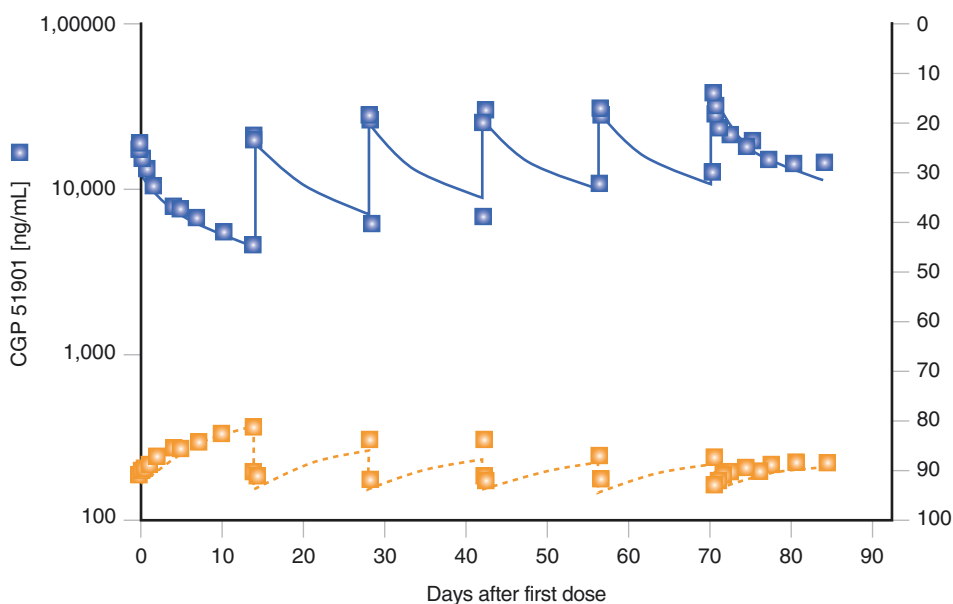
Figure 6.12 ■ Observed (Blue square) and model-predicted (Blue solid line) serum concentration of the antihuman IgE antibody CGP 51901 and observed (Orange square) and model-predicted (Orange dashed line) reduction of free IgE in one representative patient, given six IV doses of 60 mg biweekly. The predictions were modeled with a direct link PK/PD model (modified from Racine-Poon et al. 1997; with permission from Macmillan Publishers Ltd.)

mechanism (see section "Indirect Response PK/PD Models") or by a distributional delay between the drug concentrations in plasma and at the effect site.

The latter one can conceptually be described by an indirect link model, which attaches a hypothetical effect compartment to a pharmacokinetic compartment model (Fig. 6.13). The effect compartment addition to the pharmacokinetic model does not account for mass balance, i.e., no actual mass transfer is implemented in the pharmacokinetic part of the PK/PD model. Instead, drug

transfer with respect to the effect compartment is defined by the time course of the effect itself (Sheiner et al. 1979; Holford and Sheiner 1982). The effect-compartment approach, however, is necessary, as the effect site can be viewed as a small part of a pharmacokinetic compartment that from a pharmacokinetic point of view cannot be distinguished from other tissues within that compartment. The concentration in the effect compartment represents the active drug concentration at the effect site that is slowly equilibrating with the plasma and is usually linked to the effect via an E_{max} model.

Although this PK/PD model is constructed with tissue distribution as the reason for the delay of the effect, the distribution clearance to the effect compartment can be interpreted differently, including other reasons of delay, such as transduction processes and secondary post-receptor events.

Human regular U-500 insulin has recently been developed for insulin-resistant and high-dose insulin-treated patients to provide the ability of administering large doses (500 U/mL) at one-fifth the volume of that of the previously highest concentrated dosage form, human regular U-100 insulin. In order to explore the effect-time course after administration of once-daily, twice-daily and thrice-daily administration of U-500 insulin, a PK/PD model was developed based on single dose euglycemic clamp studies in healthy individuals and patients with type I diabetes. Insulin concentrations were related to glucose infusion rate as measure of pharmacodynamic effect via an effect compartment model (de la Peña et al. 2014). Model-based simulations of the different administration frequencies at steady state (Fig. 6.14) suggest that BID and TID dosing may
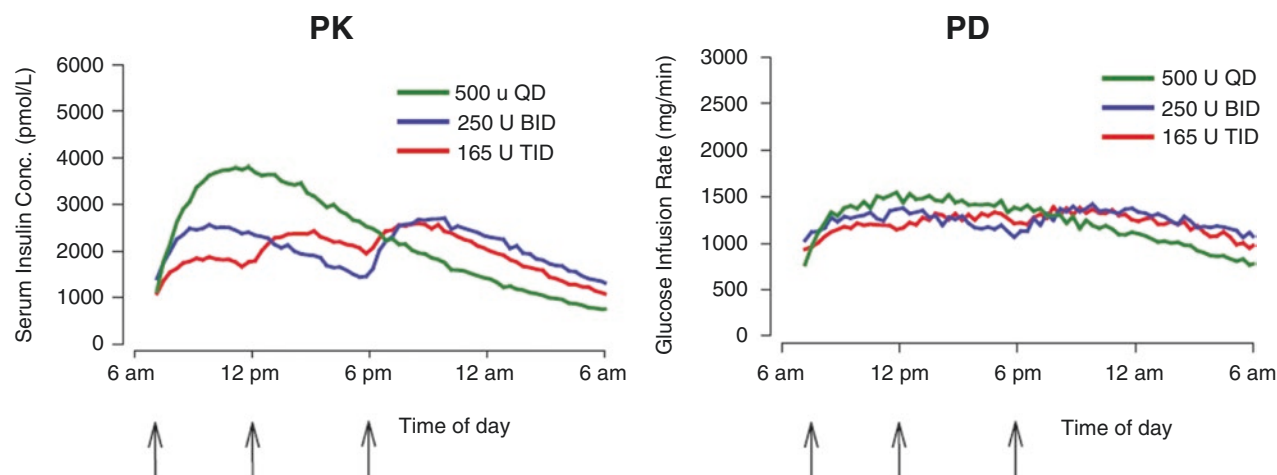

Figure 6.13 ■ Schematic of a typical indirect link PK/PD model. A hypothetical effect compartment is linked to the central compartment of a two-compartment pharmacokinetic model. The concentration in the effect compartment (C_e) drives the intensity of the pharmacodynamic effect (E) via an E_{max} relationship. CL_{1e} is the transfer clearance from the central to the effect compartment, CL_{e0} the equilibrium clearance for the effect compartment. All other PK and PD parameters are identical to those used in Fig. 6.11

Figure 6.14 ■ PK/PD model-based simulations of different dosing regimens of U-500 insulin during 24 h at steady-state: 500 U QD (*green*), 250 U BID (*blue*), 165 U TID (*red*). Arrows represent dose administration times: for QD at 7 am, BID at 7 am and 6 pm, and TID at 7 am, 12 noon, and 6 pm. The PK panel on the left shows the resulting serum insulin concentration-time profiles, the PD panel on the right side the time course of the glucose infusion rate needed to maintain euglycemia (from de la Peña et al. 2014)

provide adequate insulin action throughout the day, but QD dosing leads to fluctuations in effect that may increase the risk for hypoglycemia and may thus not be adequate for use as basal insulin therapy.

■ Indirect Response PK/PD Models

The effect of most therapeutic proteins, however, is not mediated via a direct interaction between drug concentration at the effect site and response systems but frequently involves several transduction processes that include at their rate-limiting step the stimulation or inhibition of a physiologic process, for example the synthesis or degradation of a molecular response mediator like a hormone or cytokine. In these cases, the time courses of plasma concentration and effect are also dissociated resulting in counterclockwise hysteresis for the concentration-effect relationship, but the underlying cause is not a distributional delay as for the indirect link models, but a time-consuming indirect response mechanism (Meibohm and Derendorf 1997).

Indirect response models generally describe the effect on a representative response parameter via the dynamic equilibrium between increase or synthesis and decrease or degradation of the response, with the former being a zero-order and the latter a first-order process (Fig. 6.15). The response itself can be modulated in one of four basic variants of the model. In each variant, the synthesis or degradation process of the response is either stimulated or inhibited as a function of the drug concentration. A stimulatory or inhibitory E_{max} model is used to describe the drug effect on the synthesis or degradation of the response (Dayneka et al. 1993; Sharma and Jusko 1998; Sun and Jusko 1999).

As indirect response models appreciate the underlying physiological events involved in the elaboration of the observed drug effect, their application is often preferred in PK/PD modeling as they have a mechanistic basis on the molecular and/or cellular level that often allows for extrapolating the model to other clinical situations.

An indirect response model was, for example, used in the evaluation of SB-240563, a humanized monoclonal antibody directed towards IL-5 in monkeys (Zia-Amirhosseini et al. 1999). IL-5 appears to play a significant role in the production, activation, and maturation of eosinophils. The delayed effect of SB-240563 on eosinophils is consistent with its mechanism of action via binding to and thus inactivation of IL-5. It was modeled using an indirect response model with inhibition of the production of response (eosinophil count) (Fig. 6.16). The obtained low EC_{50} value for reduction of circulating eosinophils combined with a long terminal half-life of the therapeutic protein of 13 days suggests the possibility of an infrequent dosing regimen for SB-240563 in the pharmacotherapy of dis-

Indirect response model subtypes
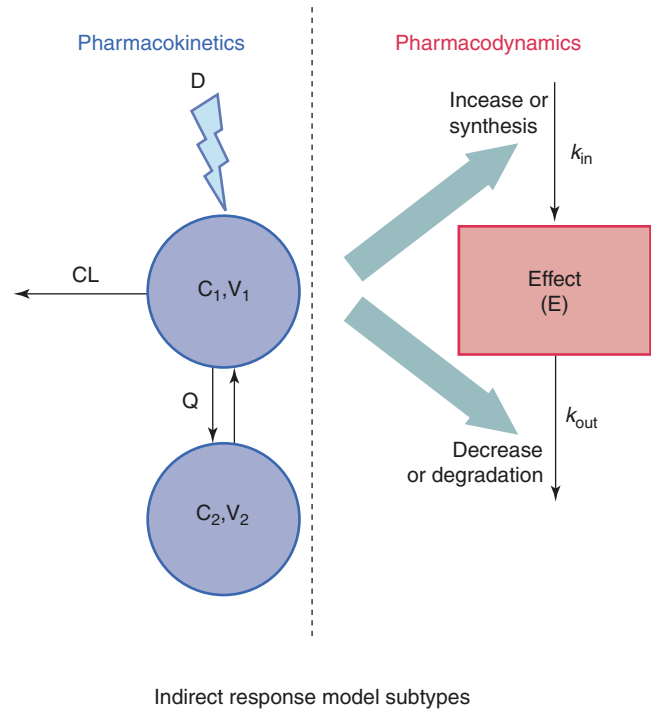
Subtype I:
inhibition of synthesis (k_{in})

$$\frac{dE}{dt} = k_{in} \cdot \left(1 - \frac{C_1}{EC_{50} + C_1}\right) - k_{out} \cdot E$$

Subtype II:
inhibition of degradation (k_{out})

$$\frac{dE}{dt} = k_{in} - k_{out} \cdot \left(1 - \frac{C_1}{EC_{50} + C_1}\right) \cdot E$$

Subtype III:
stimulation of synthesis (k_{in})

$$\frac{dE}{dt} = k_{in} \cdot \left(1 + \frac{E_{max} \cdot C_1}{EC_{50} + C_1}\right) - k_{out} \cdot E$$

Subtype IV:
stimulation of degradation (k_{out})

$$\frac{dE}{dt} = k_{in} - k_{out} \cdot \left(1 + \frac{E_{max} \cdot C_1}{EC_{50} + C_1}\right) \cdot E$$

Figure 6.15 ■ Schematic of a typical indirect response PK/PD model. The effect measure (*E*) is maintained by a dynamic equilibrium between an increase or synthesis and a decrease or degradation process. The former is modeled by a zero-order process with rate constant k_{in}, the latter by a first-order process with rate constant k_{out}. Thus, the rate of change in effect (d*E*/d*t*) is expressed as the difference between synthesis rate (k_{in}) and degradation rate (k_{out} times *E*). Drug concentration (C_1) can stimulate or inhibit the synthesis or the degradation process for the effect (*E*) via an E_{max} relationship using one of four subtypes (model I, II, III or IV) of the indirect response model. The pharmacokinetic model and all other PK and PD parameters are identical to those used in Fig. 6.11

orders with increased eosinophil function, such as asthma.

Indirect response models were also used for the effect of growth hormone on endogenous IGF-1 concentration (Sun et al. 1999), as well as the effect of epoetin-α on two response parameters, free ferritin

concentration and soluble transferrin receptor concentration (Bressolle et al. 1997). Similarly, a modified indirect response model was used to relate the concentration of the humanized anti-factor IX antibody SB-249417 to factor IX activity in Cynomolgus monkeys as well as humans (Benincosa et al. 2000; Chow et al. 2002). The drug effect in this model was introduced by interrupting the natural degradation of factor IX by sequestration of factor IX by the antibody.

■ Cell Life Span Models

A sizable number of therapeutic proteins exert their pharmacologic effect through direct or indirect modulation of blood and/or immune cell types. For these kinds of therapeutics, cell life span models have been proven useful to capture their exposure-response relationship and describe and predict drug effects (Perez-Ruixo et al. 2005). Cell life span models are mechanism-based, physiologic PK/PD models that are established based on the sequential maturation and life span-driven cell turnover of their affected cell types and progenitor cell populations. Cell life span models are especially widely used for characterizing the dose-concentration-effect relationships of hematopoietic growth factors aimed at modifying erythropoiesis, granulopoiesis, or thrombopoiesis (Perez-Ruixo et al. 2005; Agoram et al. 2006). The fixed physiologic time span for the maturation of precursor cells is the major reason for the prolonged delay between drug administration and the observed response, i.e., change in the cell count in peripheral blood. Cell life span models accommodate this sequential maturation of several precursor cell populations at fixed physiologic time intervals by a series of transit compartments linked via first- or zero-order processes with a common transfer rate constant.

A cell life span model was, for example, used to describe the effect of a multiple dose regimen of erythropoietin (EPO) 600 IU/kg given once weekly by SC injection (Ramakrishnan et al. 2004). The process of erythropoiesis and the applied PK/PD approach including a cell life span model are depicted in Figs. 6.17
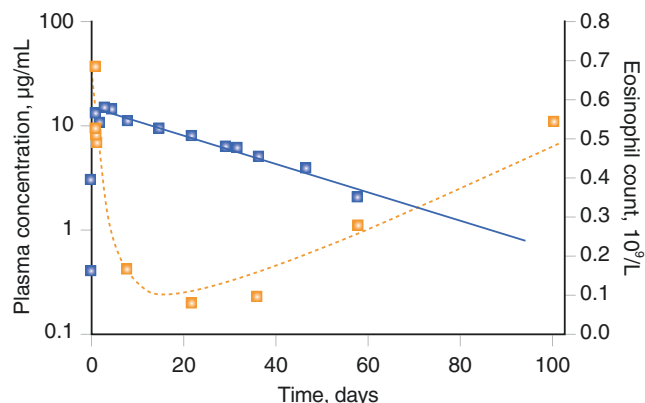
Figure 6.16 ■ Model-predicted and observed plasma concentration (observed, *blue circles*; predicted, *solid blue line*) and eosinophil count (observed, *orange squares*; predicted, *dashed orange line*) following SC administration of 1 mg/kg of the anti-IL-5 humanized monoclonal antibody SB-240563 in a Cynomolgus monkey. A mechanism-based indirect response PK/PD model was used to describe eosinophil count as a function of SB-240563 plasma concentration. The reduction in eosinophil count in peripheral blood (as effect E) was modeled as a reduction of the recruitment of eosinophils from the bone, i.e., an inhibition of the production rate k_{in} using the indirect response model of subtype I (see Fig. 6.15) (Zia-Amirhosseini et al. 1999; with permission from American Society for Pharmacology and Experimental Therapeutics)
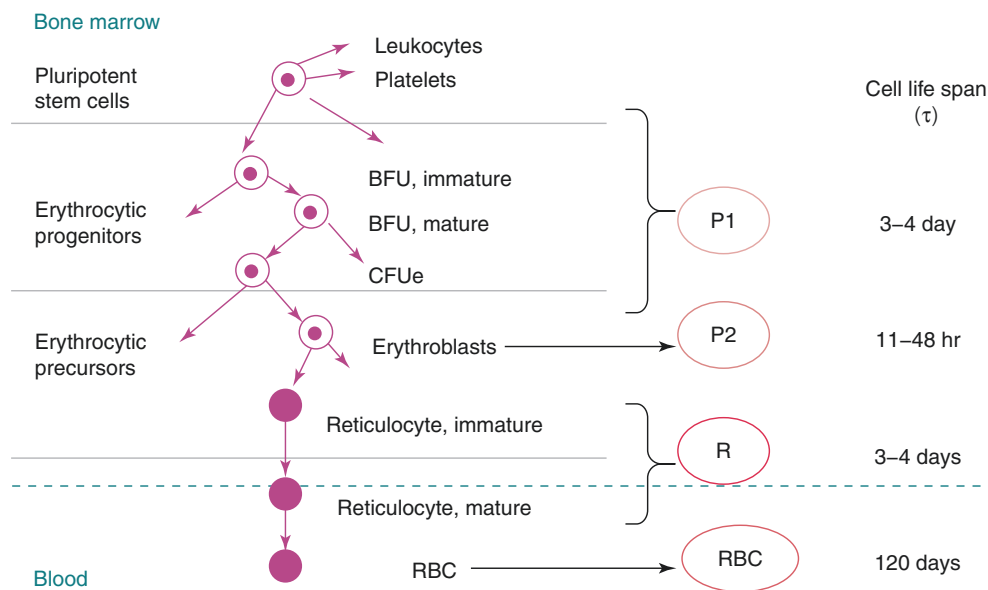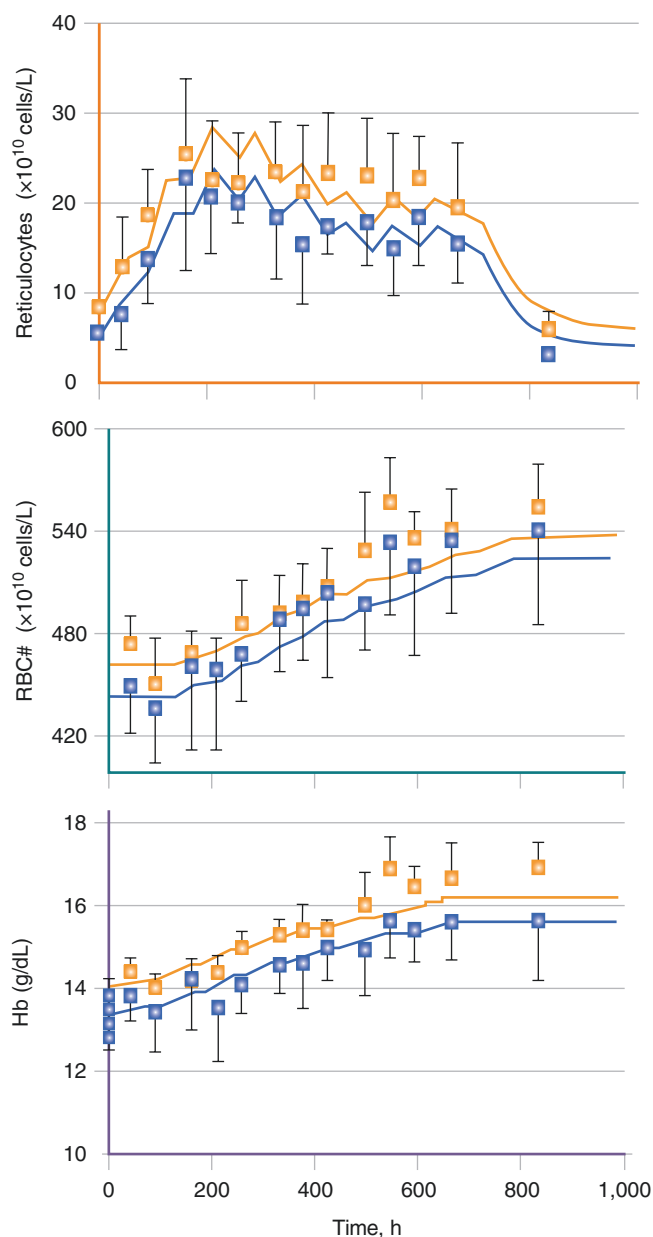
Figure 6.17 ■ Process of erythropoiesis. Erythropoietin stimulates the proliferation and differentiation of the erythrocyte progenitors (*BFU* burst-forming unit erythroid, *CFUe* colony-forming unit erythroid) as well as the erythroblasts in the bone marrow. The life spans (τ) of the various cell populations are indicated at the right (from Ramakrishnan et al. 2004, with permission from John Wiley & Sons, Inc. Copyright American College of Clinical Pharmacology 2004)

$$S(t) = 1 + \frac{E_{max} \cdot C_1}{EC_{50} + C_1}$$

$$I(t) = 1 - \frac{C_1}{IC_{50} + C_1}$$
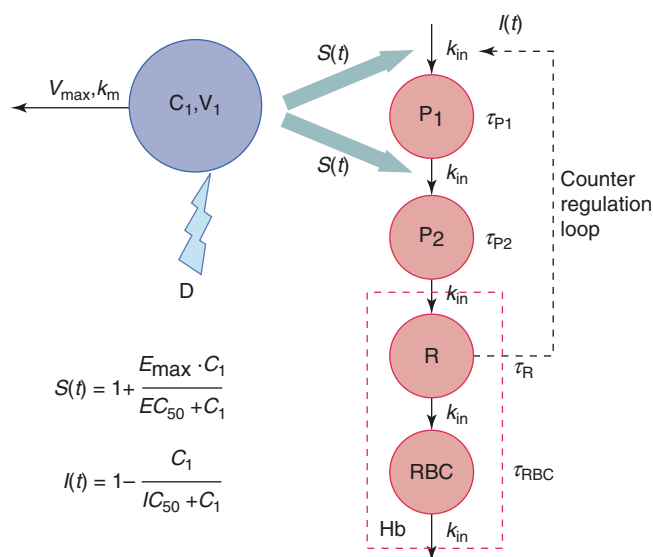
Figure 6.18 ■ A PK/PD model describing the disposition of recombinant human erythropoietin and effects on reticulocyte count, red blood cell count, and hemoglobin concentration. The PK model is a one-compartment model with Michaelis-Menten type elimination (K_m, V_{max}) from the central compartment. The PD model is a cell life span model with four sequential cell compartments, representing erythroid progenitor cells (P_1), erythroblasts (P_2), reticulocytes (R), and red blood cells (RBC). τ_{P1}, τ_{P2}, τ_R, and τ_{RBC} are the corresponding cell life spans, k_{in} the common zero-order transfer rate between cell compartments. The target parameter hemoglobin in the blood (Hb) is calculated from the reticulocyte and red blood cell count and the hemoglobin content per cell. The effect of erythropoietin is modeled as a stimulation of the production of both precursor cell populations (P_1 and P_2) in the bone marrow with the stimulation function $S(t)$. E_{max} is the maximum possible stimulation of reticulocyte production by erythropoietin, EC_{50} the plasma concentration of erythropoietin that produced half-maximum stimulation. A counter-regulatory feedback loop represents the feedback inhibition of reticulocytes on their own production by reducing the production rate of cells in the P_1 compartment via the inhibitory function $I(t)$. IC_{50} is the reticulocyte count that produced half of complete inhibition (modified from Ramakrishnan et al. 2004)

Figure 6.19 Reticulocyte, red blood cell (*RBC*), and hemoglobin (*Hb*) time courses after multiple SC dosing of 600 IU/kg/week recombinant human erythropoietin. *Orange* and *blue squares* represent data for males and females, whereas the *orange* and *blue lines* for the reticulocytes are model fittings. The *lines* in the RBC and Hb panels are the predictions using the model-fitted curves for the reticulocytes and the life span parameters (from Ramakrishnan et al. 2004, with permission from John Wiley & Sons, Inc. Copyright American College of Clinical Pharmacology 2004)
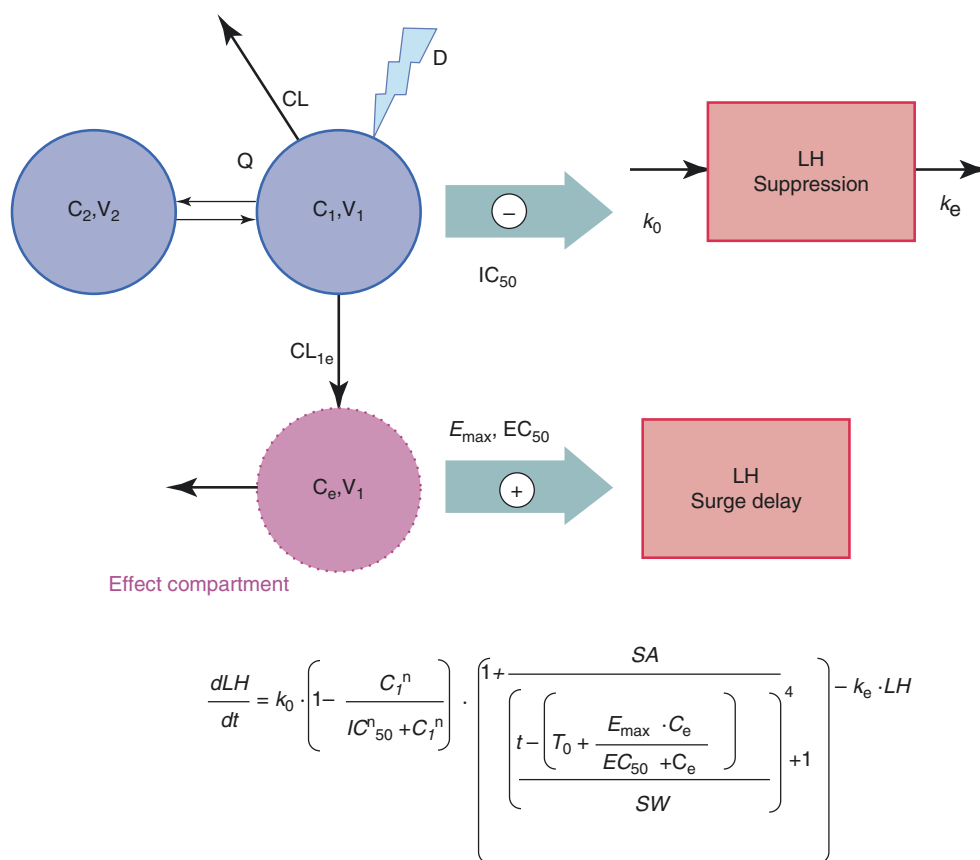
and 6.18, respectively. EPO is known to stimulate the production and release of reticulocytes from the bone marrow. The EPO effect was modeled as stimulation of the maturation of two progenitor cell populations (P1 and P2 in Fig. 6.17), including also a feedback inhibition between erythrocyte count and progenitor proliferation. Development and turnover of the subsequent populations of reticulocytes and erythrocytes was modeled, taking into account their life spans as listed in Fig. 6.17. The hemoglobin concentration as pharmacodynamic target parameter was calculated from erythrocyte and reticulocyte counts and hemoglobin content per cell. Figure 6.19 shows the resulting time courses in reticulocyte count, erythrocyte count, and hemoglobin concentration.

■ Complex Response Models

Since the effect of most therapeutic proteins is mediated via complex regulatory physiologic processes including feedback mechanisms and/or tolerance phenomena, some PK/PD models that have been described

$$\frac{dLH}{dt} = k_0 \cdot \left(1 - \frac{C_1^{\,n}}{IC_{50}^{\,n} + C_1^{\,n}}\right) \cdot \left(1 + \frac{SA}{\left(\dfrac{t - \left(T_0 + \dfrac{E_{max} \cdot C_e}{EC_{50} + C_e}\right)}{SW}\right)^4 + 1}\right) - k_e \cdot LH$$

Figure 6.20 ■ A PK/PD model for the combined effect of the luteinizing hormone-releasing hormone (LH-RH) antagonist cetrorelix on luteinizing hormone (*LH*) suppression and delay of the LH surge. The PK model is a two-compartment model identical to the model described in Fig. 6.11. The PD model consists of two components: an indirect response model of subtype I to model the suppression of LH by cetrorelix and an indirect link model that models the delay in LH surge as a function of the cetrorelix concentration in a hypothetical effect compartment. Both PD model components are combined in the provided mathematical expression that describes the rate of change in LH concentration (dLH/d*t*) as a function of both processes. LH is the LH concentration, k_0 and k_e are the zero-order production rate and first-order elimination rate constants for LH at baseline, C_1 and C_e are the cetrorelix concentrations in plasma and a hypothetical effect compartment, respectively, SA is the LH surge amplitude, *t* is the study time in terms of cycle day, T_0 is the study time in terms of cycle day at which the peak occurs under baseline conditions, SW is the width of the peak in time units, IC_{50} is the cetrorelix concentration that suppresses LH levels by 50%, n is the Hill factor fixed at 2, E_{max} is the maximum delay in LH surge, and EC_{50} is the cetrorelix concentrations that produces half of E_{max}. Baseline data analysis indicated that the slope of the surge peak and SW were best fixed at values of 4 and 24 h, respectively. Other PK and PD parameters are identical to those used in Fig. 6.13 (modified from Nagaraja et al. 2000)

for protein drugs are much more sophisticated than the four classes of models previously discussed.

One example of such a complex modeling approach has been developed for the therapeutic effects of the luteinizing hormone-releasing hormone (LH-RH) antagonist cetrorelix (Nagaraja et al. 2000, 2003; Pechstein et al. 2000). Cetrorelix is used for the prevention of premature ovulation in women undergoing controlled ovarian stimulation in in vitro fertilization protocols. LH-RH antagonists suppress the LH levels and delay the occurrence of the preovulatory LH surge, and this delay is thought to be responsible for postponing ovulation. The suppression of LH was modeled in the PK/PD approach with an indirect-response model approach directly linked to

cetrorelix plasma concentrations (Fig. 6.20) (Nagaraja et al. 2003). The shift in LH surge was linked to cetrorelix concentration with a simple E_{max} function via a hypothetical effect compartment to account for a delay in response via complex signal transduction steps of unknown mechanism of action. Figure 6.21 shows the application of this PK/PD model to characterize the LH suppression and LH surge delay after subcutaneous administration of cetrorelix to groups of 12 women at different dose levels. The analysis revealed a marked dose–response relationship for the LH surge and thus predictability of drug response to cetrorelix (Nagaraja et al. 2000).

Another example for a complex PK/PD model is the cytokinetic model used to describe the effect of
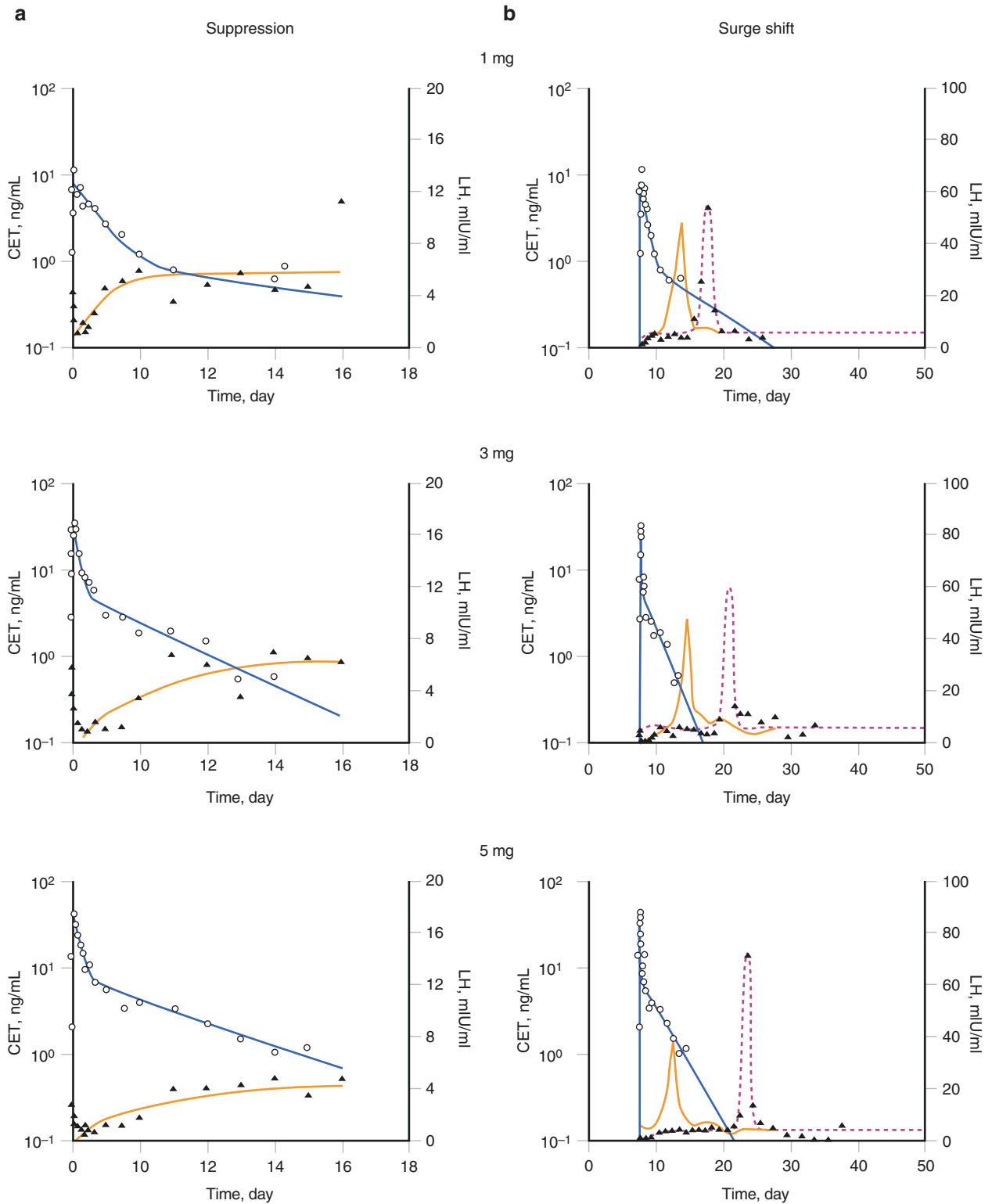
Figure 6.21 ■ Pharmacokinetic and pharmacodynamic relationship between cetrorelix (CET;○) and LH concentrations (▲) after single doses of 1, 3, and 5 mg cetrorelix in representative subjects. Cetrorelix and LH concentrations were modeled using the PK/PD model presented in Fig. 6.20. *Left panel*: LH suppression. *Right panel*: LH suppression and LH surge profiles. The *solid blue line* represents the model-fitted cetrorelix concentration, the *dashed purple line* the model-fitted LH concentration, and the *solid orange line* in the *right panels* the pretreatment LH profile (not fitted). The cetrorelix-dependent delay in LH surge is visible as the rightward shift of the LH surge profile under cetrorelix therapy compared to the respective pretreatment LH profile (from Nagaraja et al. 2000, with permission from Macmillan Publishers Ltd.)
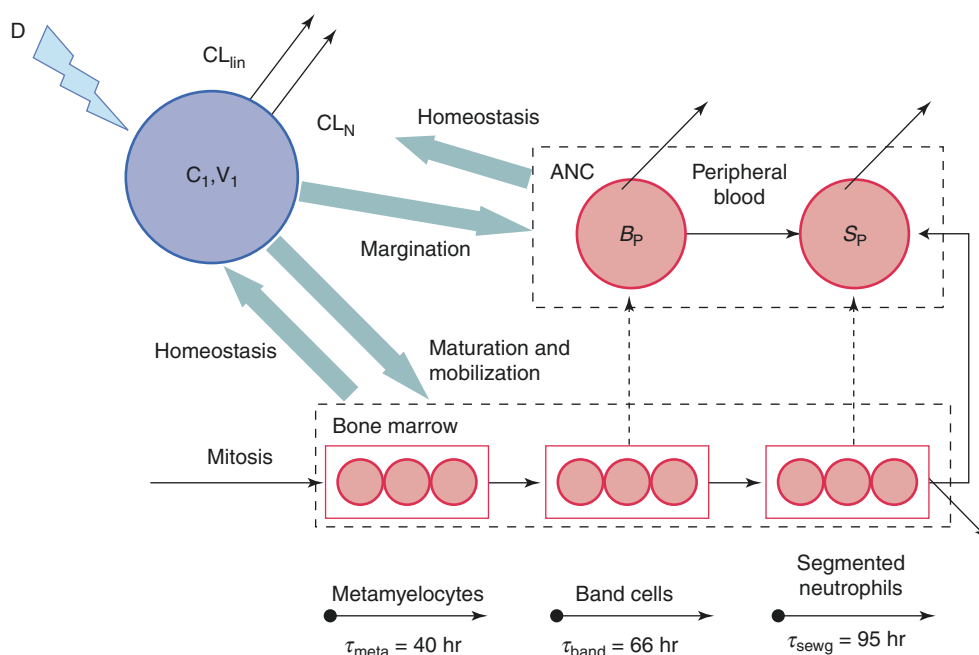
Figure 6.22 ■ A PK/PD model describing the granulopoietic effects of pegfilgrastim. The PK model is a one-compartment model with two parallel elimination pathways, a first-order elimination process (CL_{lin}) and a neutrophil-mediated elimination process (CL_N). C_1 and V_1 are the concentrations in the PK compartment and the corresponding volume of distribution. The PD model is a cytokinetic model similar to the cell life span model in Fig. 6.18. Three maturation stages of neutrophils and their respective life spans (t_{meta}, t_{band}, t_{seg}) are included in the model, metamyelocytes, band cells, and segmented neutrophils. Each maturation stage is modeled by three sequential transit compartments. Serum concentrations of pegfilgrastim stimulate mitosis and mobilization of band cells and segmented neutrophils in bone marrow, decrease maturation times for postmitotic cells in marrow, and affect margination of the peripheral blood band cell (B_P) and segmented neutrophil (S_P) populations, the sum of which is the total absolute neutrophil count (*ANC*). Changes in neutrophil counts in peripheral blood provide feedback regulation of pegfilgrastim clearance (modified from Roskos et al. 2006)

pegfilgrastim on the granulocyte count in peripheral blood (Roskos et al. 2006; Yang 2006). Pegfilgrastim is a PEGylated form of the human granulocyte colony-stimulating factor (G-CSF) analogue filgrastim. Pegfilgrastim, like filgrastim and G-CSF, stimulates the activation, proliferation, and differentiation of neutrophil progenitor cells and enhances the functions of mature neutrophils (Roskos et al. 2006). Pegfilgrastim is mainly used as supportive care to ameliorate and enhance recovery from neutropenia secondary to cancer chemotherapy regimens. As already discussed in the section on PEGylation, pegfilgrastim follows target-mediated drug disposition with saturable receptor-mediated endocytosis by neutrophils as major elimination pathway (CL_N) and a parallel first-order process as minor elimination pathway (CL_{lin}; Fig. 6.22). The clearance for the receptor-mediated pathway is determined by the absolute neutrophil count (ANC), the sum of the peripheral blood band cell (B_p) and segmented neutrophil (S_p) populations.

A maturation-structured cytokinetic model of granulopoiesis was established to describe the relationship between pegfilgrastim serum concentration

and neutrophil count (Fig. 6.22). The starting point is the production of metamyelocytes from mitotic precursors. Subsequent maturation stages are captured as band cells and segmented neutrophils in the bone marrow. Each maturation stage is modeled by three sequential transit compartments. Pegfilgrastim concentrations are assumed to increase ANC by stimulating mitosis and mobilization of band cells and segmented neutrophils from the bone marrow into the systemic circulation. Pegfilgrastim also promotes rapid margination of peripheral blood neutrophils, i.e., adhesion to blood vessels; this effect is modeled as an expansion of neutrophil dilution volume.

Figure 6.23 shows observed and modeled pegfilgrastim concentration-time and ANC-time profiles after escalating single SC dose administration of pegfilgrastim. The presented PK/PD model for pegfilgrastim allowed determining its EC_{50} for the effect on ANC. Based on this EC_{50} value and the obtained pegfilgrastim plasma concentrations, it was concluded that a 100 μg/kg dose was sufficient to reach the maximum therapeutic effect of pegfilgrastim on ANC (Roskos et al. 2006; Yang 2006).
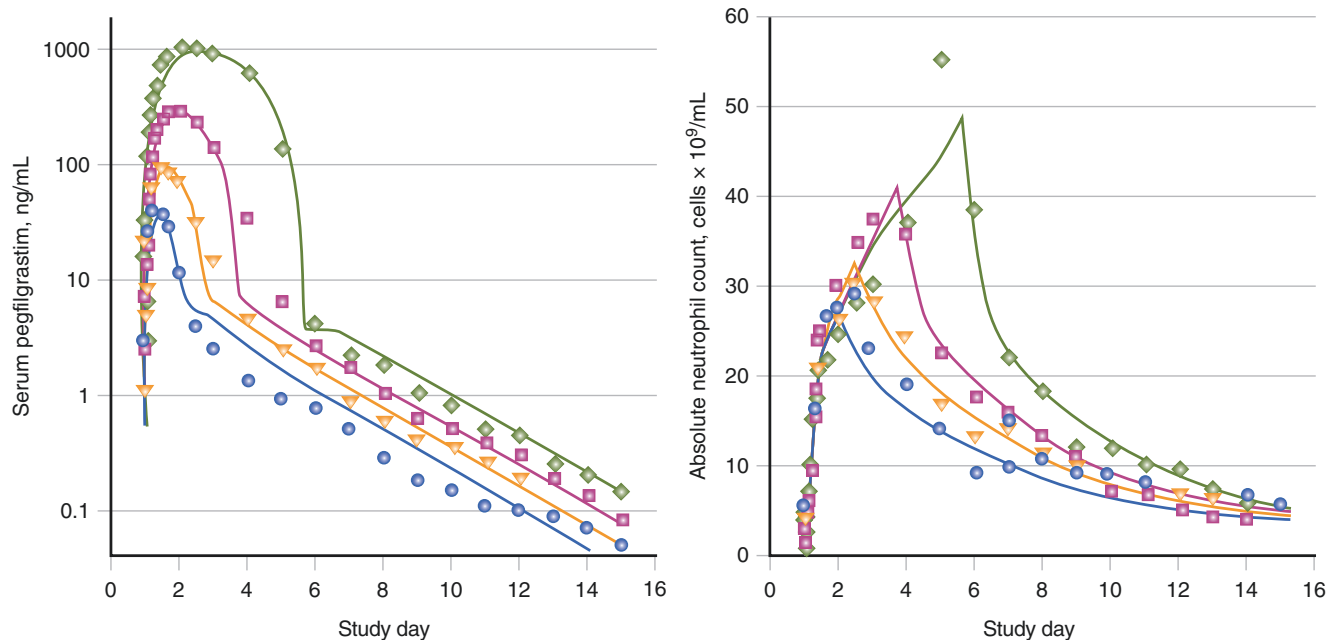
Figure 6.23 ■ Pegfilgrastim concentration time course and absolute neutrophil count (ANC) time profiles in healthy subjects after a single SC administration of 30, 60, 100, and 300 µg/kg pegfilgrastim (*n* = 8/dose group). Measured data are presented by symbols as mean ± SEM. Lines represent modeled time courses based on the cytokinetic PK/PD model presented in Fig. 6.22 (from Roskos et al. 2006, with permission from John Wiley & Sons, Inc. Copyright American College of Clinical Pharmacology 2006)

CONCLUSION

The pharmacokinetic and pharmacodynamic characteristics of peptides and proteins form the basis for their therapeutic application. Appreciation of the pharmacokinetic and pharmacodynamic differences between therapeutic biologics and traditional small-molecule drugs will empower the drug development scientist as well as the healthcare provider to handle, evaluate, and apply these compounds in an optimal fashion during the drug development process as well as during applied pharmacotherapy. Rationale, scientifically based drug development and pharmacotherapy based on the use of pharmacokinetic and pharmacodynamic concepts will undoubtedly propel the success and future of therapeutic proteins and might ultimately contribute to provide the novel medications that may serve as the key for the aspired "precision medicine" in the healthcare systems of the future (Dugger et al. 2018).

SELF-ASSESSMENT QUESTIONS

■ Questions

1. What are the major elimination pathways for protein drugs after administration?
2. Which pathway of absorption is rather unique for proteins after SC injection?
3. What is the role of plasma binding proteins for natural proteins?
4. How do the sugar groups on glycoproteins influence hepatic elimination of these glycoproteins?
5. In which direction might elimination clearance of a protein drug change when antibodies against the protein are produced after chronic dosing with the protein drug? Why?
6. What is the major driving force for the transport of proteins from the vascular to the extravascular space?
7. Why are therapeutic proteins generally not active upon oral administration?
8. Many therapeutic proteins exhibit Michaelis-Menten type, saturable elimination kinetics. What are the underlying mechanisms for this pharmacokinetic behavior?
9. Explain counterclockwise hysteresis in plasma concentration-effect plots.
10. Why is mechanism-based PK/PD modeling a preferred modeling approach for therapeutic proteins?

■ Answers

1. Proteolysis, glomerular filtration followed by intraluminal metabolism or tubular reabsorption with intracellular lysosomal degradation, receptor-

mediated endocytosis followed by metabolism in the skin, muscle, liver and possibly other organs and tissues.

2. Biodistribution from the injection site into the lymphatic system.

3. Plasma proteins may act as circulating reservoirs for the proteins that are their ligands. Consequently, the protein ligands may be protected from elimination and distribution.

4. In some cases, the sugar groups are recognized by hepatic receptors (e.g., galactose by the galactose receptor), facilitating receptor-mediated uptake and metabolism.

5. Clearance may increase or decrease by forming antibody-protein complexes. A decrease of clearance occurs when the antibody-protein complex is eliminated slower than free protein. An increase of clearance occurs when the protein-antibody complex is eliminated more rapidly than the unbound protein, such as when reticuloendothelial uptake is stimulated by the complex.

6. Protein extravasation, i.e., transport from the blood or vascular space to the interstitial tissue space, is predominantly mediated by convection. Protein molecules follow the fluid flux from the vascular space through pores between adjacent cells into the interstitial space. Drainage of the interstitial space through the lymphatic system allows therapeutic proteins to distribute back into the vascular space.

7. The gastrointestinal mucosa is a major absorption barrier for hydrophilic macromolecule such as proteins. In addition, therapeutic peptides and proteins are degraded by the extensive peptidase and protease activity in the gastrointestinal tract. Both processes minimize the oral bioavailability of therapeutic proteins.

8. Receptor-mediated endocytosis is the most frequent cause of nonlinear pharmacokinetics in therapeutic proteins. Its occurrence becomes even more prominent if the therapeutic protein undergoes target-mediated drug disposition, i.e., if the receptor-mediated endocytosis is mediated via the pharmacologic target of the therapeutic protein. As the binding to the target is usually of high affinity, and the therapeutic protein is often dosed to saturate the majority of the available target receptors for maximum pharmacologic efficacy, saturation of the associated receptor-mediated endocytosis as elimination pathway is frequently encountered.

9. Counterclockwise hysteresis is an indication of the indirect nature of the effects seen for many protein drugs. It can be explained by delays between the appearance of drug in plasma and the appearance of the pharmacodynamic response. The underlying cause may either be a distributional delay between the drug concentrations in plasma and at the effect site (modeled with an indirect link PK/PD model) or by time-consuming post-receptor events that cause a delay between the drug-receptor interaction and the observed drug effect, for example, the effect on a physiologic measure or endogenous substance.

10. Therapeutic proteins are often classified as "targeted therapies" where the drug compound acts on one specific, well-defined response pathway. This well-documented knowledge on the mechanism of action can relatively easily be translated into a mechanism-based PK/PD modeling approach that incorporates the major physiological processes relevant for the pharmacologic effect. The advantage of mechanism-based as compared to empirical PK/PD modeling is that mechanism-based models are usually more robust and allow more reliable simulations beyond the actually measured data.

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