6 Pharmacokinetics and Pharmacodynamics of Therapeutic Proteins and Nucleic Acids

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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, which 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 nucleic acid-based therapeutics 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 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

This chapter highlights some of the major pharmacokinetic properties and processes relevant for the majority of proteins and provides examples of well-characterized pharmacodynamic relationships for protein drugs. It also briefly discusses the pharmacokinetic characteristics of nucleotide-based therapeutics as emerging new group of biotechnology products, even though they are usually chemically synthesized rather than produced in living organisms. The clinical pharmacology of monoclonal antibodies (mAbs), including special aspects in their pharmacokinetics and pharmacodynamics, is 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 Suggested Reading).

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D. J. A. Crommelin et al. (eds.), *Pharmaceutical Biotechnology*, https://doi.org/10.1007/978-3-031-30023-3_6

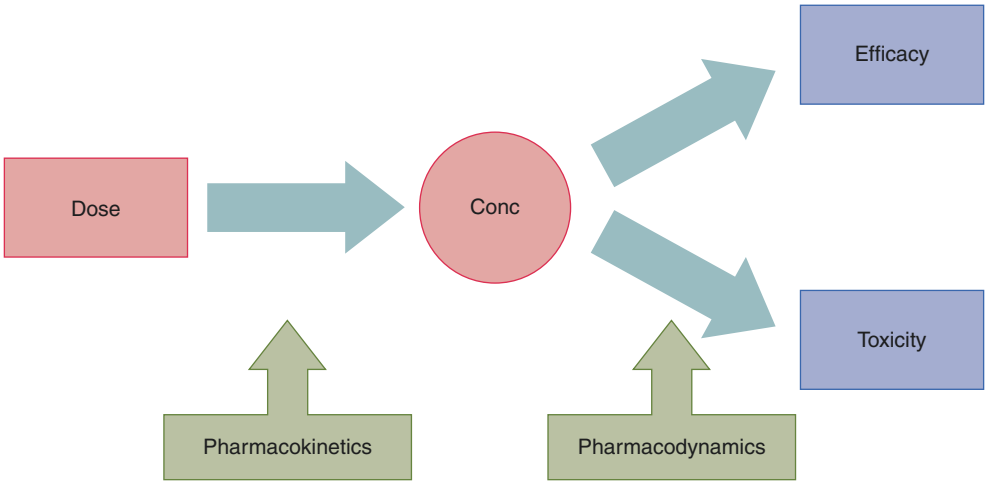


Fig. 6.1 The central paradigm of clinical pharmacology: the dose–concentration–effect relationship

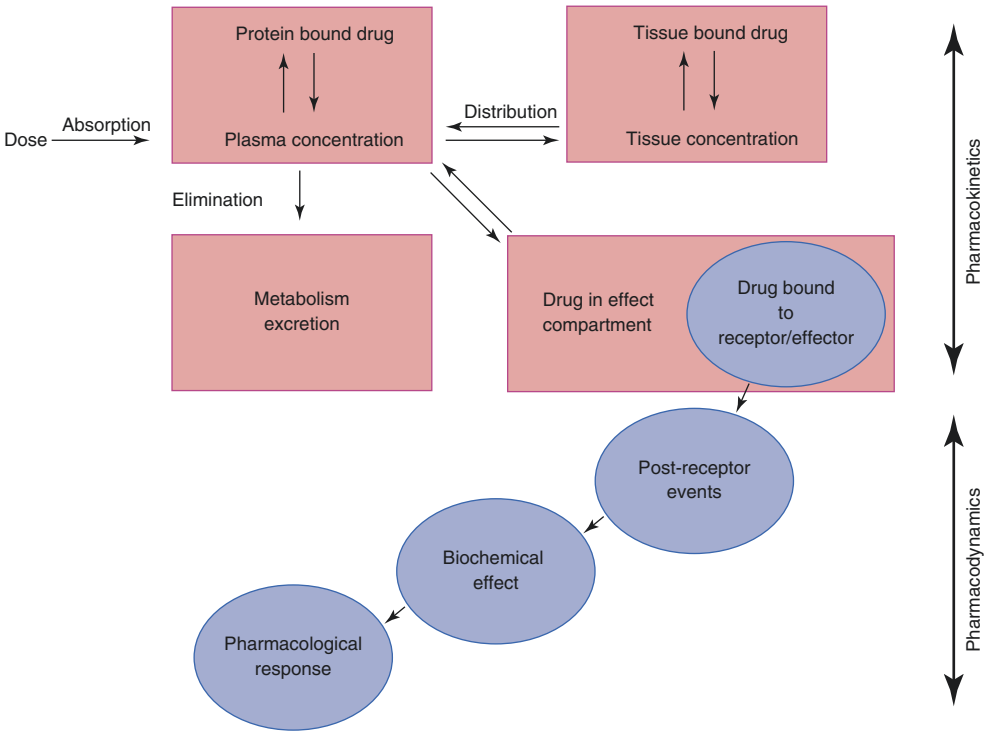


Fig. 6.2 Physiological scheme of pharmacokinetic and pharmacodynamic processes

Pharmacokinetics of Therapeutic Proteins

The in vivo disposition of protein drugs may often be predicted to a large degree from their physiological function (Tang and Meibohm 2006). Small proteins and 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, large 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 2006). 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

Therapeutic 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 make it the most efficient body compartment for protein metabolism. Furthermore, the gastrointestinal mucosa presents a major absorption barrier for water-soluble macromolecules such as 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; Verma et al. 2021). 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 proteins are discussed in Chap. 5.

Parenteral Administration

Most 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 nonoral administration pathways are utilized, including nasal, buccal, rectal, vaginal, transdermal, ocular, and pulmonary drug delivery (see Chap. 5).

IV administration of 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 study comparing SC versus IV administration of epoetin-α in patients receiving hemodialysis reported 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. Thus, numerous mAbs that had initially been brought to market as IV dosage forms have more recently been extended to also offer SC dosage forms. One of the related challenges beyond dosage volume limitations and resulting formulation issues is the translation of body weight-based dosing for the IV dosage form to fixed dosing as required by the SC route (Yapa et al. 2016).

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. For many subcutaneously administered mAbs; however, SC bioavailability is in the range of 50–80% (Ryman and Meibohm 2017).

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 prior to entering the blood stream, 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 and lymph 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 mAbs 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. 5.15) (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 between vascular and interstitial space (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. 16), while this fraction is approaching 100% for mAbs (150 kDa).

For mAbs 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 mAb 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 mAbs, 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. A related model-based analysis suggests that lymphatic flow rate is the most influential factor for t_{max} of SC administered mAbs (Zhao et al. 2013).

Preferential uptake into lymphatic vessels after SC administration is of particular importance for those agents that target 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).

Beyond molecular weight and size, 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 mAbs (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 distri-

bution 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 that 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.

After IV administration, 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 mAb specific to the CD4 molecule on the surface of T lymphocytes (Mould et al. 1999). Similarly, secukinumab, a human mAb 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 to 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

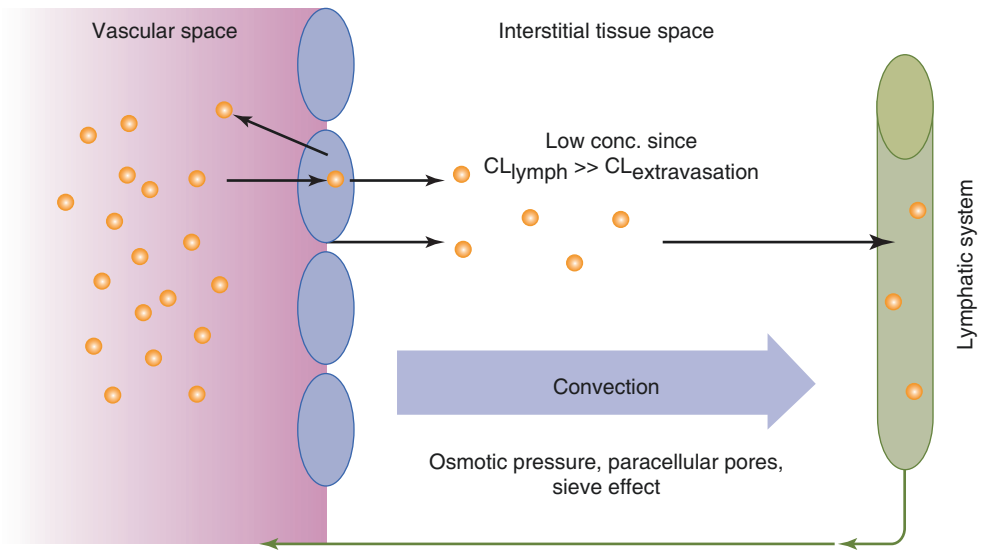


Fig. 6.3 Distribution mechanisms of therapeutic proteins: convective extravasation rather than diffusion as major distribution process. $CL_{extravasation}$ transfer clearance from the vascular to the interstitial space, CL_{lymph} transfer clearance from the interstitial space to the lymphatic system

initial volume of distribution (Colburn 1991; Dirks and Meibohm 2010). An example for such a distribution pattern is the t-PA analog 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 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 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 proteins is binding to endogenous protein structures. Physiologically active endogenous 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 elimi-

nation 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 a membrane-standing receptor 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 the mAb alemtuzumab (Albitar et al. 2004).

Apart from this specific binding, peptides and proteins may also be nonspecifically bound to plasma proteins. For example, metkephamid, a met-enkephalin analog, was described to be 44–49% bound to albumin (Taki et al. 1998), and octreotide, a somatostatin analog, 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 Protein Metabolism").

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. Nonmetabolic 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 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 proteins in this context describes the irreversible removal of active substance from the vascular space, which includes besides metabolism also cellular uptake. Thus, intracellular uptake is per se more an elimination rather than a distribution process (Tang and Meibohm 2006). 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 within subcellular compart-

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

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

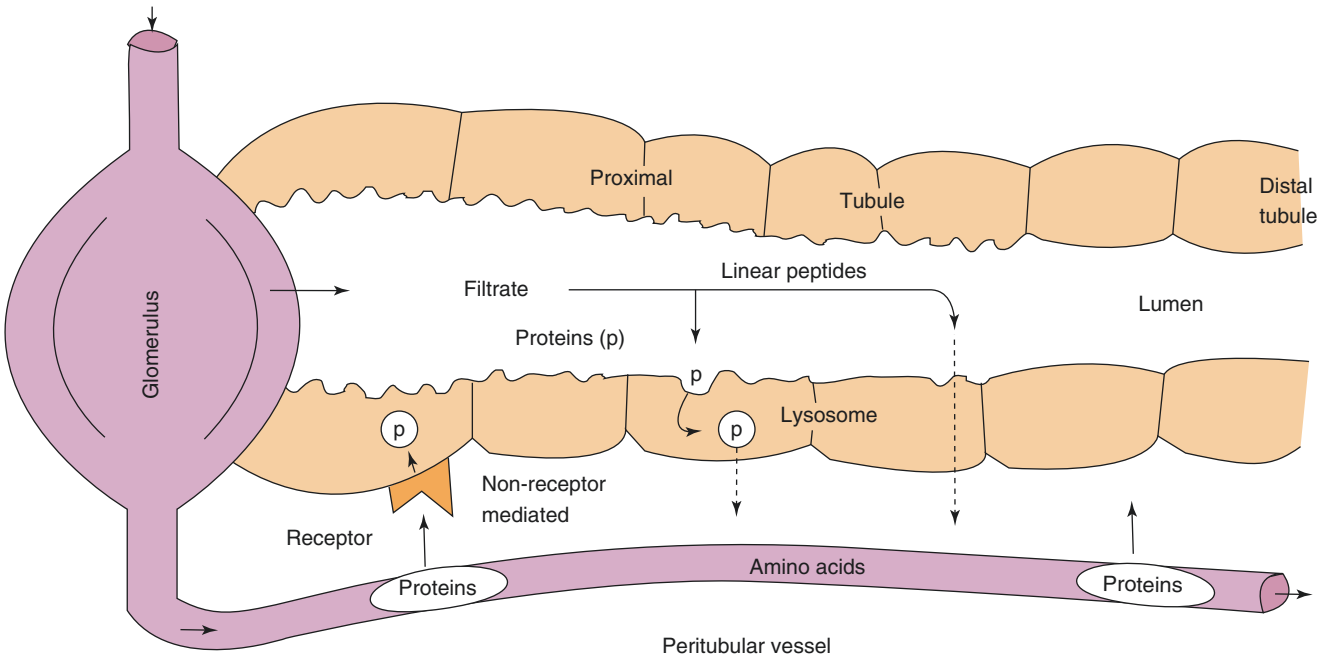


Fig. 6.4 Pathways of renal metabolism of peptides and proteins: glomerular filtration followed by either (I) intraluminal metabolism or (II) tubular reabsorption with intracellular lysosomal metabolism and (III) peritubular extraction with intracellular lysosomal metabolism. (Modified from Maack et al. (1985))

ments such as lysosomes. Thus, intracellular uptake is the rate limiting step for nonspecific, proteolytic clearance of therapeutic proteins. This uptake occurs either by pinocytosis, a fluid-phase endocytosis, or by a receptor-mediated endocytosis. Pinocytosis is a relatively unspecific and inefficient endocytic process by endothelial cells lining the blood and lymphatic vessels. In pinocytosis, protein molecules are taken up into cells by forming invaginations of cell membrane around extracellular fluid droplets that are subsequently taken up as membrane vesicles. Due to the large surface area of endothelial cells in the body (>1000 m^2), the process can despite its inefficiency substantially contribute to the elimination of therapeutic proteins. Nonspecific proteolytic degradation following pinocytotic uptake is thus not limited to a specific organ but occurs throughout the body, particularly in those organs and tissues rich in capillary beds with endothelial cells. Thus, the skin, muscles, and the gastrointestinal tract are the major elimination organs for the nonspecific proteolytic degradation of many therapeutic proteins, including immunoglobulin G-based therapeutics such as mAbs and antibody derivatives. In addition, the phagocytic cells of the reticuloendothelial system have been identified as a major contributor to the unspecific proteolytic degradation of many therapeutic proteins (Ryman and Meibohm 2017). Receptor-mediated endocytosis processes are more relevant for specific organs and tissues and will be discussed in the subsequent sections.

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 proteins may also be metabolized in the endothelial cells lining the vast capillary beds of the gastrointestinal tract as well as in resident phagocytic cells. 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.

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). 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 filtrates (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 mAb Fab fragment (48 kDa) idarucizumab. The likely underlying mechanism is temporary saturation of the promiscuous endocytic receptors, megalin and cubilin, on the apical membrane of renal tubular cells that facilitate endocytic uptake of proteins from the tubular lumen (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 degradation of 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 mAbs (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 post-glomerular 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 nonspecific proteolytic clearance via endothelial cells and the reticuloendothelial system, as well as renal and gastrointestinal metabolism, the liver may also play a major role in the metabolism of some 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 in the liver as in any other cells in the body is the active uptake of proteins into the different liver cell types as these protein molecules are unable to passively cross cell membranes due to their high molecular weight and charge. Uptake of larger

peptides and proteins can either be facilitated through pinocytosis as described above or by receptor-mediated endocytosis.

Receptor-mediated endocytosis is usually 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 membrane-standing receptor proteins. The receptors are usually integral membrane glycoproteins with an exposed binding domain on the extracellular side of the cell membrane. Many different receptor systems use this same clathrin-mediated endocytosis process. After the binding of the circulating protein to the receptor, the complex is already present or moves to clathrin-coated pit regions in the cell membrane, and the membrane invaginates and pinches off to form an endocytotic coated vesicle that contains the receptor and ligand. This process is referred to as internalization of the drug-receptor complex. 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 oftentimes 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). MAb-based therapeutics usually consist of a heterogeneous mixture of different glycoforms based on the glycan chains attached to amino acid Asn297 on each heavy chain. Some of these glycoforms that have a high content of mannose (Man5, Man8, Man9) have been described to exhibit a three times faster clearance compared to other glycan structures, presumably via interaction with the hepatic mannose receptor (Falck et al. 2021). Similarly, the asialoglycoprotein receptor recognizes glycosylated proteins with terminal galactose and galactose derivatives and has been implicated in the rapid clearance of erythropoietin, reteplase, lanoteplase, and clotting factor VIII (Lunghi et al. 2022).

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 toward 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,

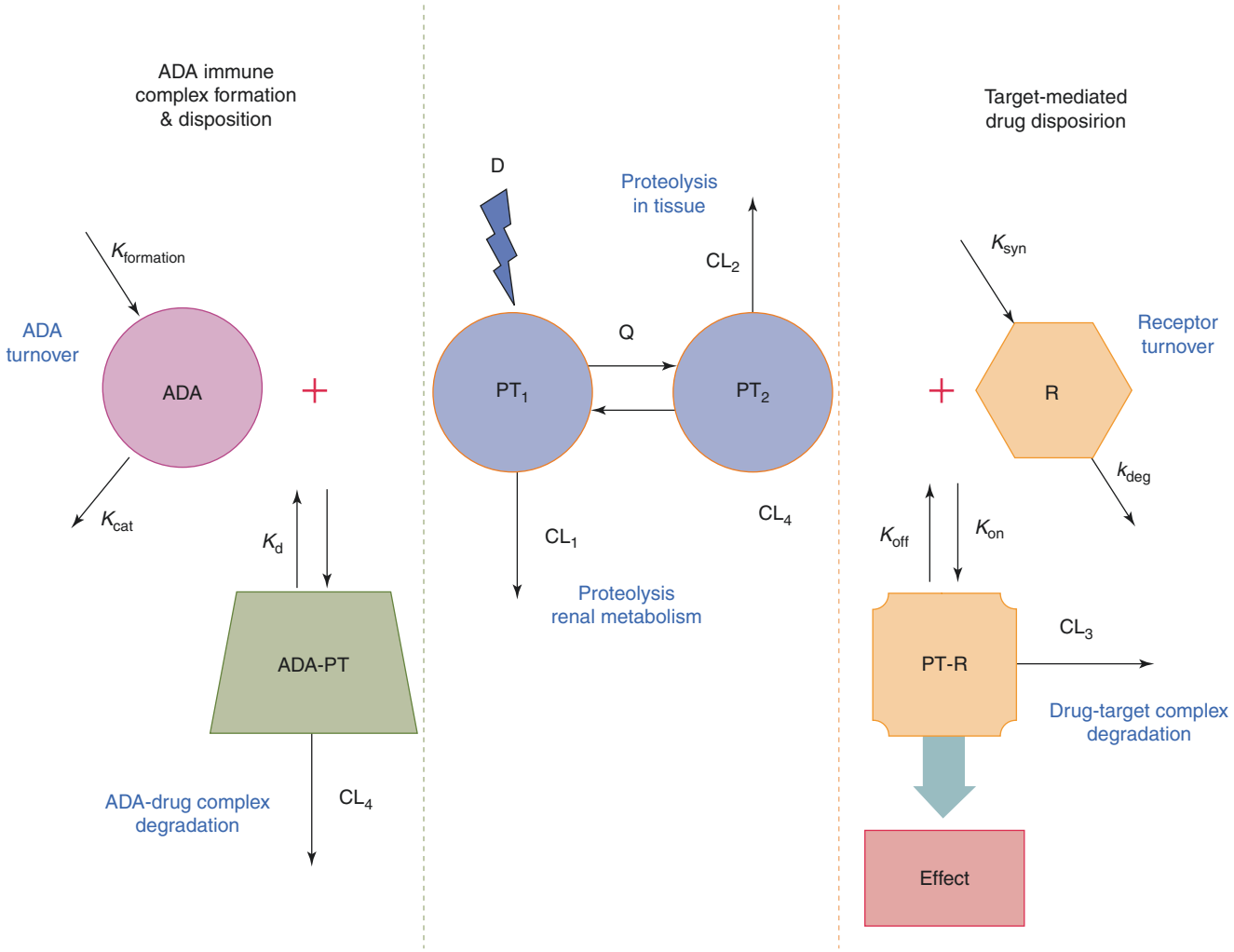


Fig. 6.5 Example of multiple clearance pathways affecting the pharmacokinetics of a typical therapeutic protein. Depicted is a two-compartment pharmacokinetic model with intravenous administration of a dose (*D*), concentrations of the therapeutic protein in the central (PT_1) and peripheral (PT_2) compartment, 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 antidrug 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-receptor 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))

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

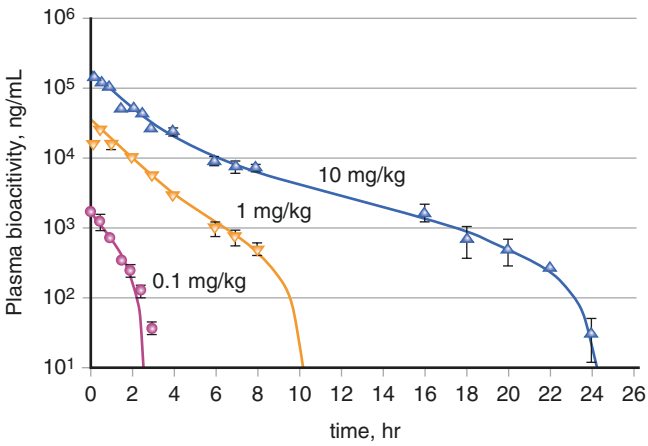


Fig. 6.6 Nonlinear pharmacokinetics of macrophage colony-stimulating factor (M-CSF), presented as measured (*triangles* and *circles*; mean ± SE) and modeled plasma bioactivity–time curves (*lines*) after intravenous injection of 0.1 mg/kg ($n = 5$), 1.0 mg/kg ($n = 3$), and 10 mg/kg ($n = 8$) in rats. Bioactivity is used as a substitute for concentration. (From Bauer et al. (1994), with permission from American Society for Pharmacology and Experimental Therapeutics)

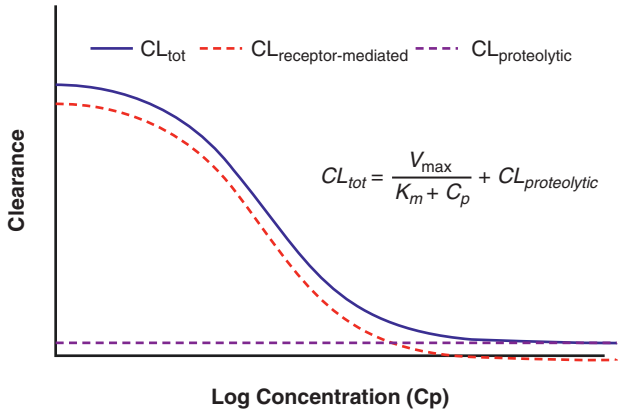


Fig. 6.7 Conceptualization of the concentration-dependent changes in clearance for a therapeutic protein that undergoes receptor- (or target-) mediated elimination displayed in a semi-logarithmic plot. The therapeutic protein is assumed to be eliminated by two parallel clearance processes, one linear, nonsaturable process with relatively low efficiency such as nonspecific proteolytic clearance ($CL_{proteolytic}$), and a second nonlinear, saturable process characterized by Michaelis–Menten-type kinetics and high efficiency at low concentrations such as a receptor- or target-mediated clearance process ($CL_{receptor\text{-}mediated} = V_{max}/[K_m + Cp]$). The total clearance ($CL_{tot}$) for the therapeutic protein is the sum of the clearances for both pathways. At low concentration, the total clearance is fast and dominated by the target mediated elimination, and the contribution of the nonspecific proteolytic pathway is limited to a low level. With increasing drug plasma concentrations, the receptor-mediated elimination pathway becomes increasingly saturated once the drug concentrations are in the range of or larger than the K_m value for this pathway. Consequently, the total clearance progressively decreases. At very high drug concentrations relative to K_m, the receptor-mediated clearance asymptotically reaches 0, and the total clearance is only determined by the nonspecific proteolytic clearance. V_{max} maximum clearance rate [amount/time]; K_m Michaelis–Menten constant: concentration [amount/volume] at 50% of V_{max}, *Cp* plasma concentration

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- and concentration-dependent and decreases with increasing dose or concentration. 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, all M-CSF elimination pathways are active and unsaturated, while at high concentrations nonrenal elimination pathways are saturated resulting in nonlinear pharmacokinetic behavior (Fig. 6.6) (Bauer et al. 1994).

The concentration-dependent change in clearance for therapeutic proteins undergoing receptor-mediated elimination is conceptualized in Fig. 6.7. Nonlinearity in pharmacokinetics resulting from target-mediated drug disposition has also been observed for numerous mAbs, for instance for the anti-EGFR chimeric mAb cetuximab in patients with head-and-neck cancer (Dirks et al. 2008) and the antiproprotein convertase subtilisin/kexin type 9 (PCSK9) mAb evolocumab in patients with hypercholesterolemia (Gibbs et al. 2017). For cetuximab, increasing concentrations lead to the saturation of the available EGFR molecules expressed in the vascular space, the primary distribution space of the mAb, thereby saturating this target-mediated clearance pathway. Similarly, for evolocumab, increasing doses and their corresponding concentrations led to the saturation of the available target PCSK9 in liver, kidneys, and small intestine, resulting in an over-proportional increase in exposure with increasing doses.

Modulation of Protein Disposition by the FcRn Receptor

Immunoglobulin G (IgG)-based mAbs 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 across the placenta via transcytosis. 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 cell types. This is facilitated by intercepting IgG in the endosomes via a pH-dependent binding process and recy-

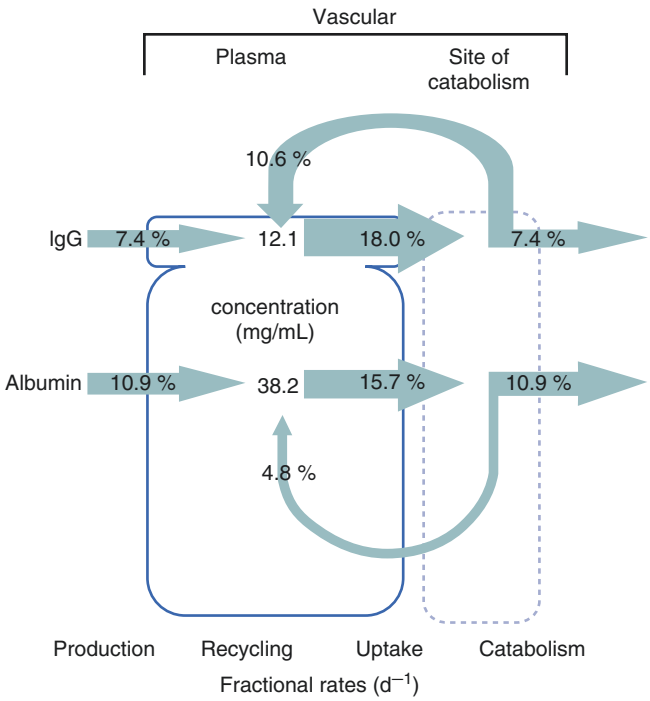


Fig. 6.8 Effect of FcRn-mediated recycling on IgG and albumin turnover in humans expressed as fractional rates. Shown are homeostatic plasma concentrations (12.1 and 38.2 mg/mL), fractional catabolic rates (7.4 and 10.9%/day), the FcRn-mediated fractional recycling rates (10.6 and 4.8%/day), and the 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)

cling it to the systemic circulation (Wang et al. 2008). The interaction with the FcRn receptor thereby prolongs the elimination half-life of 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 with only very weak binding 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. 6.8. 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 antidrug 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 recombinant human interferon-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 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. 6.9). 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 mAbs (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

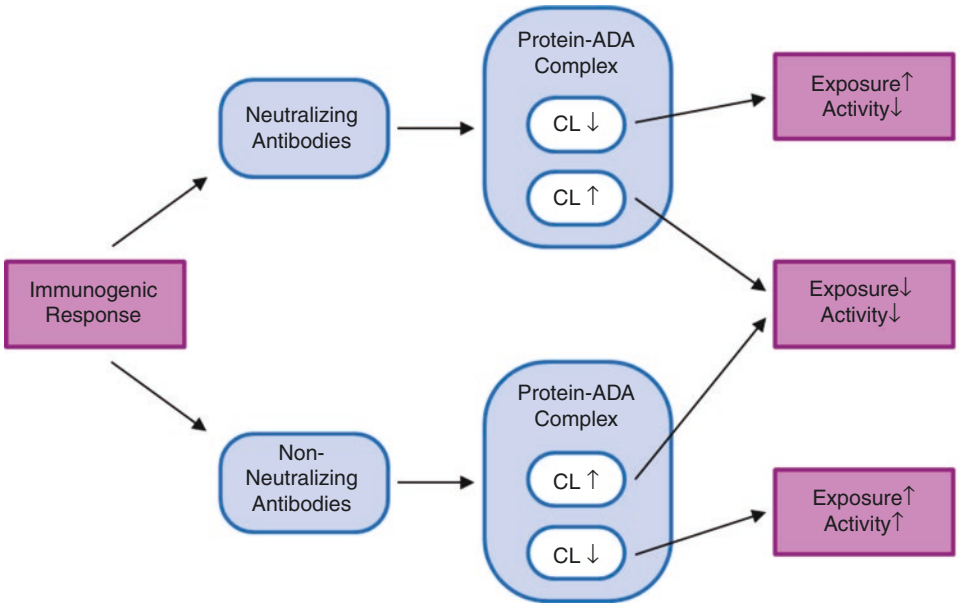


Fig. 6.9 Effect of antidrug antibody (ADA) formation on the pharmacokinetics and pharmacodynamics of therapeutic proteins. *CL* clearance

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 mAbs 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

Proteins often exhibit distinct species specificity with regard to structure and activity. 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 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 biological rates (i.e., clearance, flow rates), 1 for volumes of distribution, 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 6.10, for example, shows allometric plots for the clearance and volume of distribution of a 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).

More recent work on scaling the pharmacokinetics of mAbs 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). Especially allometric extrapolation of pharmacokinetic parameters from mice to humans for mAbs has been challenging because murine FcRn has a substantially higher binding affinity to human IgG molecules compared to human FcRn (Ober et al. 2001). Thus, preclinical pharmacokinetic experiments in mice with humanized or human mAbs result in arbitrarily low and thus overly optimistic nonspecific proteolytic clearance values in mice that when allometrically scaled largely underestimate human clearance. Transgenic animal models such as the Tg32 and Tg276 mouse models that express the human instead of the murine FcRn are increasingly used to circumvent this problem but are also challenged by other resulting effects such as arbitrarily low endogenous immunoglobulin levels (Ko et al. 2021).

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

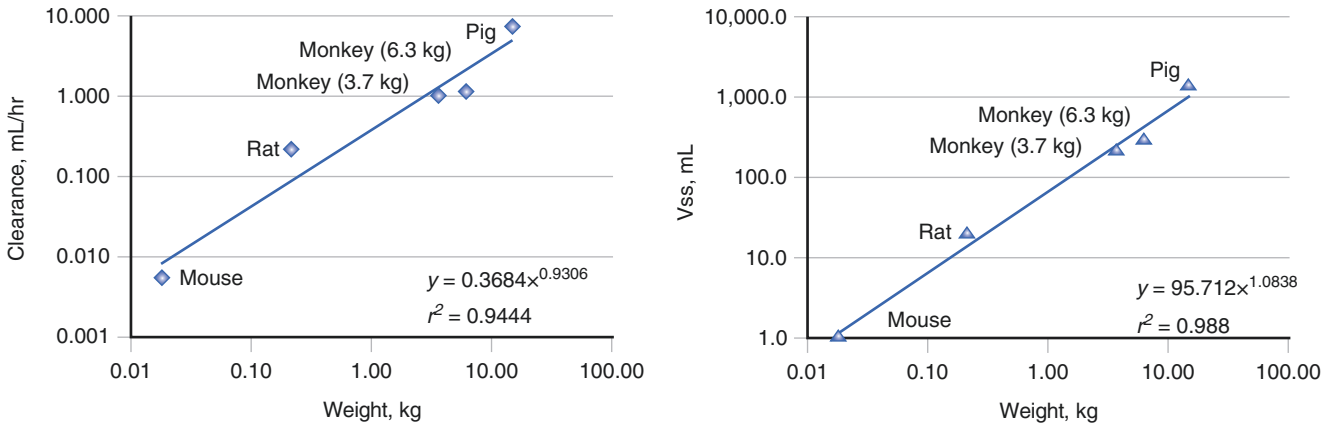


Fig. 6.10 Allometric plots of the pharmacokinetic parameter clearance and volume of distribution at steady state (V_{ss}) for the P-selectin antagonist rPSGL-Ig. Each data point within the plot represents an averaged value of the respective pharmacokinetic parameter in one of five species: mouse, rat, 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)

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-human 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 2013; 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 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 toward 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. 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 toward L-asparaginase using PEG conjugation techniques (Graham 2003). Pegaspargase is well-tolerated compared to L-asparaginase, with only 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 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 analog 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. 17.

Pharmacodynamics of Therapeutic Proteins

Therapeutic proteins are usually highly potent compounds with steep dose–effect curves as they are targeted therapies toward 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; Mould and Meibohm 2016). Combination of pharmacodynamics with pharmacokinetics by integrated

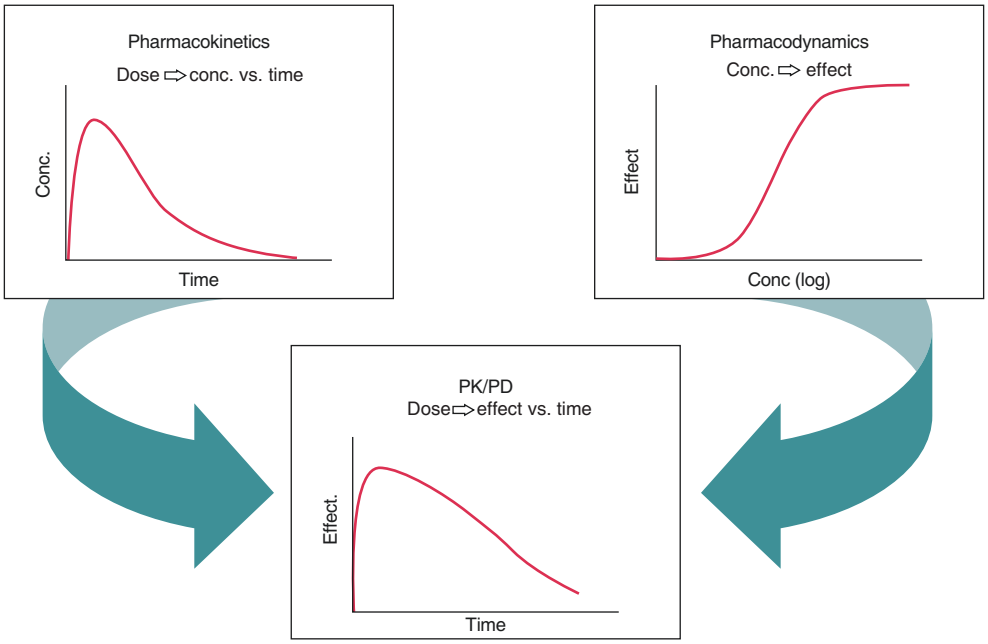


Fig. 6.11 General concept of PK/PD modeling. Pharmacokinetic-pharmacodynamic (PK/PD) modeling combines a pharmacokinetic model component that describes the time course of drug in plasma and a pharmacodynamic model component that relates the plasma concentration to the drug effect in order to describe the time course of the effect intensity resulting from the administration of a certain dosage regimen. (From Derendorf and Meibohm (1999))

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. 6.11) (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 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 (EfpiaMidWorkgroup et al. 2016), 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; Liu et al. 2021).

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; Zhu et al. 2019). Thus, PK/PD concepts and model-informed 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 (also referred to as turnover 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 (Lee et al. 2003).

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. 6.12). The most frequently used direct link pharmacodynamic model is a sigmoid E_{max} model:

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

with E_{max} as maximum achievable effect, Cp 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).

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. 6.13) (Racine-Poon et al. 1997). It should be noted that the peak and trough concentrations and effects

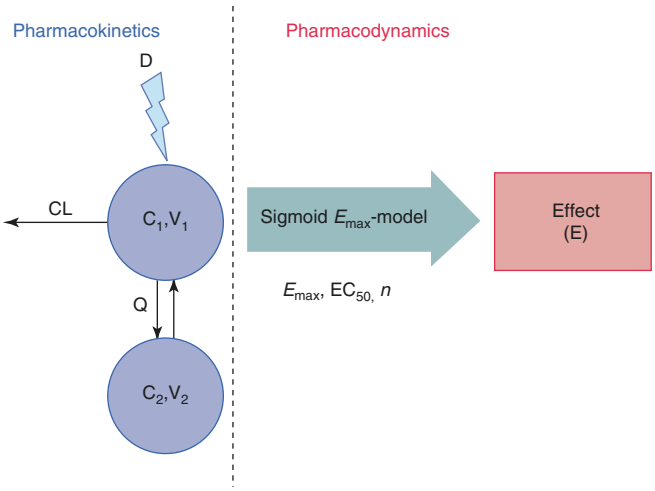


Fig. 6.12 Schematic of a typical direct link PK/PD model. The PK model is a typical two-compartment model with a linear elimination clearance from the central compartment (CL) and a distributional clearance (Q). C_1 and C_2 are the concentrations in the central and peripheral compartments, and V_1 and 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

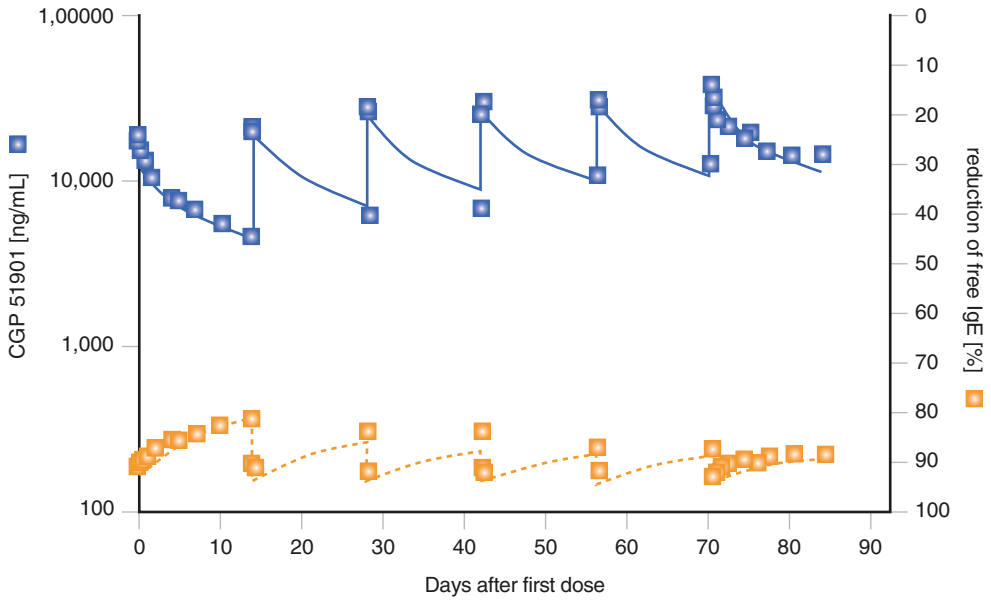


Fig. 6.13 Observed (■) and model-predicted (——) serum concentration of the antihuman IgE antibody CGP 51901 and observed (■) and model-predicted (- - - -) reduction of free IgE in one representative patient, given six IV doses of 60 mg biweekly. The predictions were modeled with a direct link PK/PD model. (Modified from Racine-Poon et al. (1997); with permission from Macmillan Publishers Ltd.)

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 mechanism (see next section) or by a distributional delay between the drug concentrations in plasma and at the effect site.

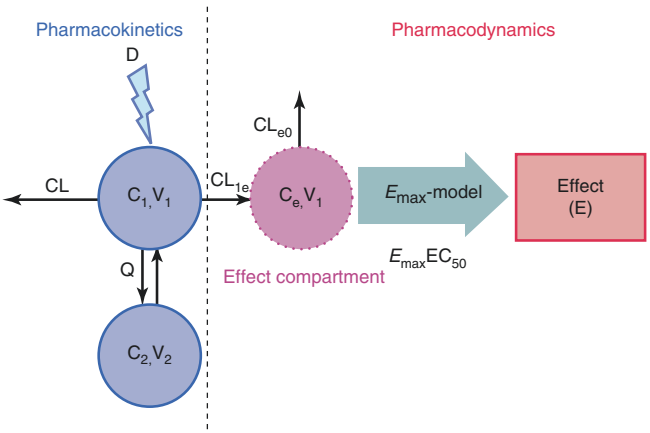


Fig. 6.14 Schematic of a typical indirect link PK/PD model. A hypothetical effect compartment is linked to the central compartment of a two-compartment pharmacokinetic model. The concentration in the effect compartment (C_e) drives the intensity of the pharmacodynamic effect (E) via an E_{max} relationship. 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. 6.12

The latter one can conceptually be described by an indirect link model, which attaches a hypothetical effect compartment to a pharmacokinetic compartment model (Fig. 6.14). 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 pharmacodynamics effect via an effect compartment model (de la Pena et al. 2014). Model-based simulations of the different administration frequencies at steady state (Fig. 6.15) suggest that BID and TID dosing may 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. 6.16). 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 effect site 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).

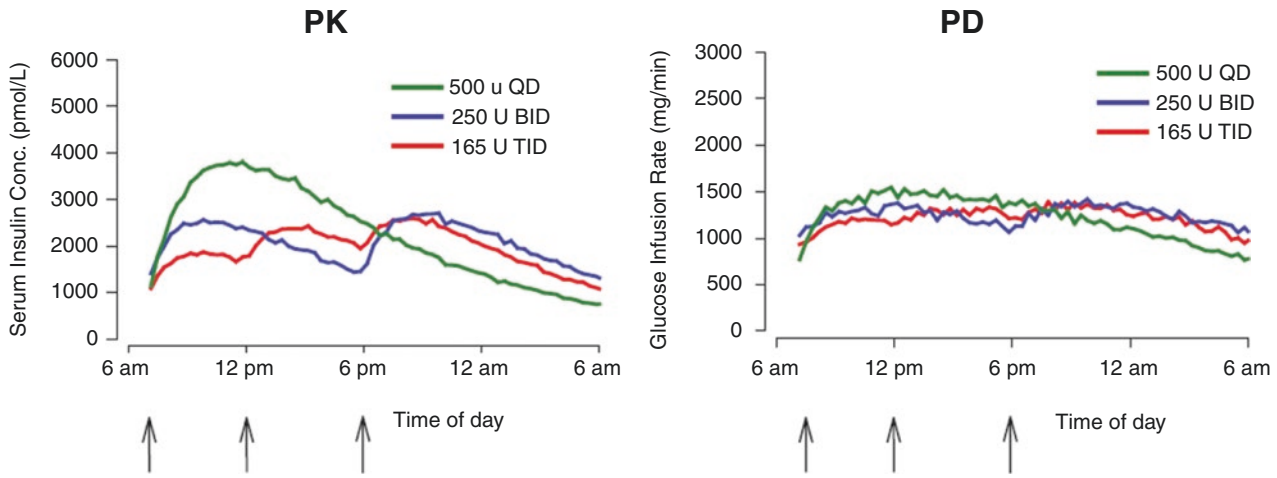
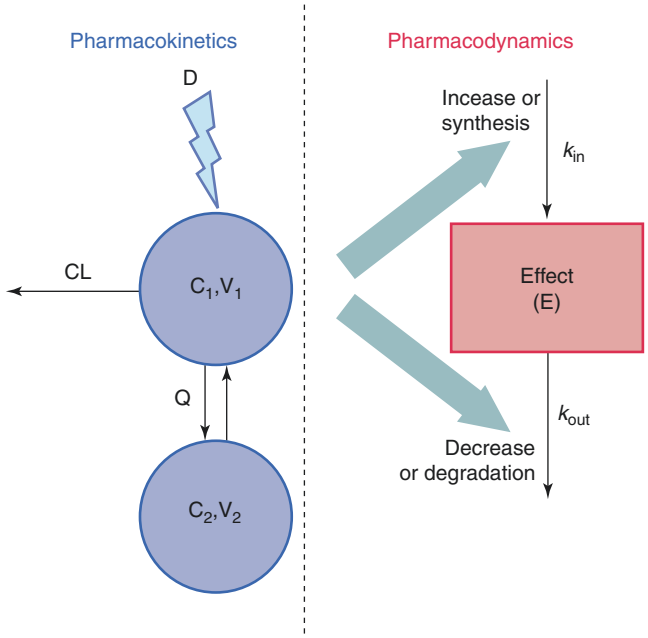


Fig. 6.15 PK/PD model-based simulations of different dosing regimens of U-500 insulin during 24 h at steady-state: 500 U QD (*green*), 250 U BID (*blue*), 165 U TID (*red*). Arrows represent dose administration times: for QD at 7 am, BID at 7 am and 6 pm, and TID at 7 am, 12 noon, and 6 pm. The PK panel 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))



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})

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

Fig. 6.16 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 (dE/dt) 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. 6.12

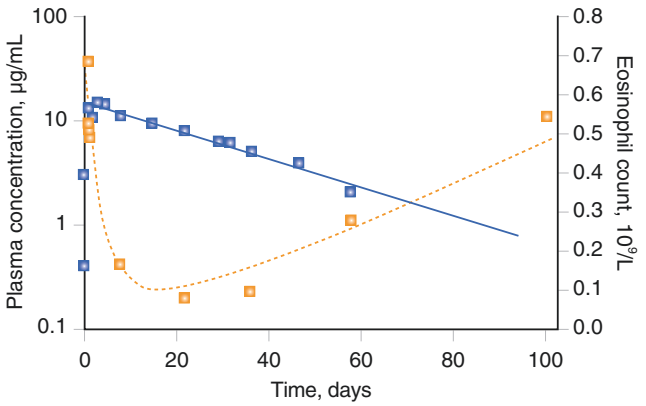


Fig. 6.17 Model-predicted and observed plasma concentration (observed, *blue squares*; predicted, *solid blue line*) and eosinophil count (observed, *orange squares*; predicted, *dashed orange line*) following SC administration of 1 mg/kg of the anti-IL-5 humanized monoclonal antibody SB-240563 in a 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. 6.16). (Zia-Amirhosseini et al. (1999); with permission from American Society for Pharmacology and Experimental Therapeutics)

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 mAb 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. 6.17). 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 disorders 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), for the effect of epoetin-α on two response parameters, free ferritin concentration, and soluble transferrin receptor concentration (Bressolle et al. 1997) as well as for the effect of the alirocumab, a proprotein convertase subtilisin kexin type 9 (PCSK9) targeting mAb, on low-density lipoprotein cholesterol (Nolain et al. 2022). 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 relationship 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 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. 6.18 and 6.19, respectively. Erythropoietin is known to stimulate the production and release of reticulocytes from the bone marrow. The erythropoietin effect was modeled as stimulation of the maturation of two progenitor cell populations (P1 and P2 in Fig. 6.18), 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. 6.18. The hemoglobin concentration as pharmacodynamic target parameter was calculated from erythrocyte and reticulocyte counts and hemoglobin content per cell. Figure 6.20 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 for protein drugs are much more sophisticated than the four classes of models previously discussed.

One example of such a complex modeling approach is the cytokinetic model used to describe the effect of 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) analog 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 and a parallel first-order process as minor elimination pathway (Fig. 6.21). The clearance for the receptor-mediated pathway is determined by the absolute neutrophil count (ANC), the sum of the peripheral blood band cell, and segmented neutrophil populations.

A maturation-structured cytokinetic model of granulopoiesis was established to describe the relationship between pegfilgrastim serum concentration and neutrophil count (Fig. 6.21). 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 6.22 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).

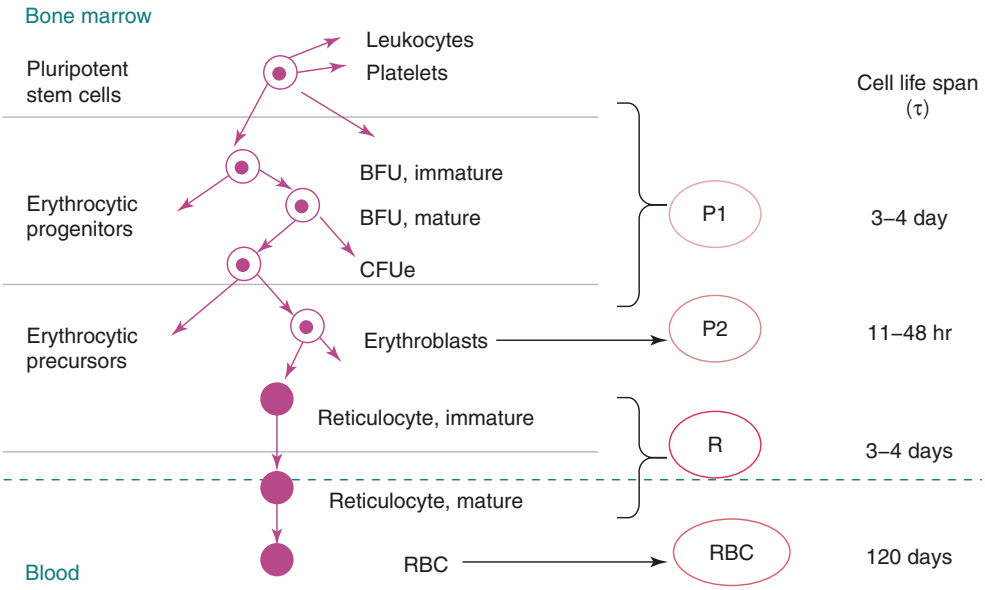
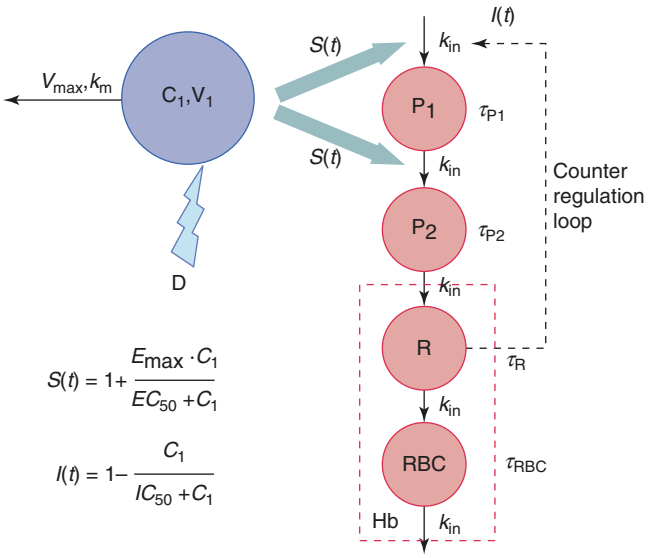


Fig. 6.18 Process of erythropoiesis. Erythropoietin stimulates the proliferation and differentiation of the erythrocyte progenitors (*BFU* burst-forming unit erythroid, *CFUe* colony-forming unit erythroid), as well as the erythroblasts in the bone marrow. The life spans (τ) of the various cell populations are indicated at the right. (From Ramakrishnan et al. (2004), with permission from John Wiley & Sons, Inc. Copyright American College of Clinical Pharmacology 2004)



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

$$I(t) = 1 - \frac{C_1}{IC_{50} + C_1}$$

Fig. 6.19 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))

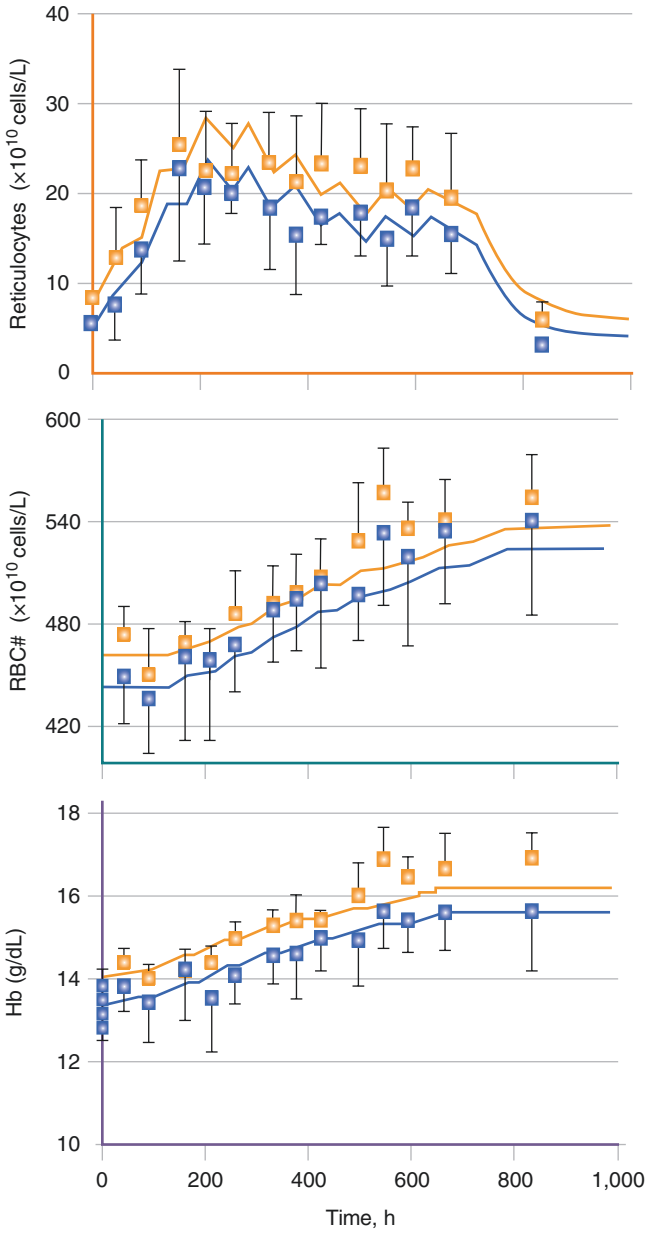


Fig. 6.20 Reticulocyte, red blood cell (*RBC*), and hemoglobin (*Hb*) time courses after multiple SC dosing of 600 IU/kg/week recombinant human erythropoietin. *Orange* and *blue squares* represent data for males and females, whereas the *orange* and *blue lines* for the reticulocytes are model fittings. 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)

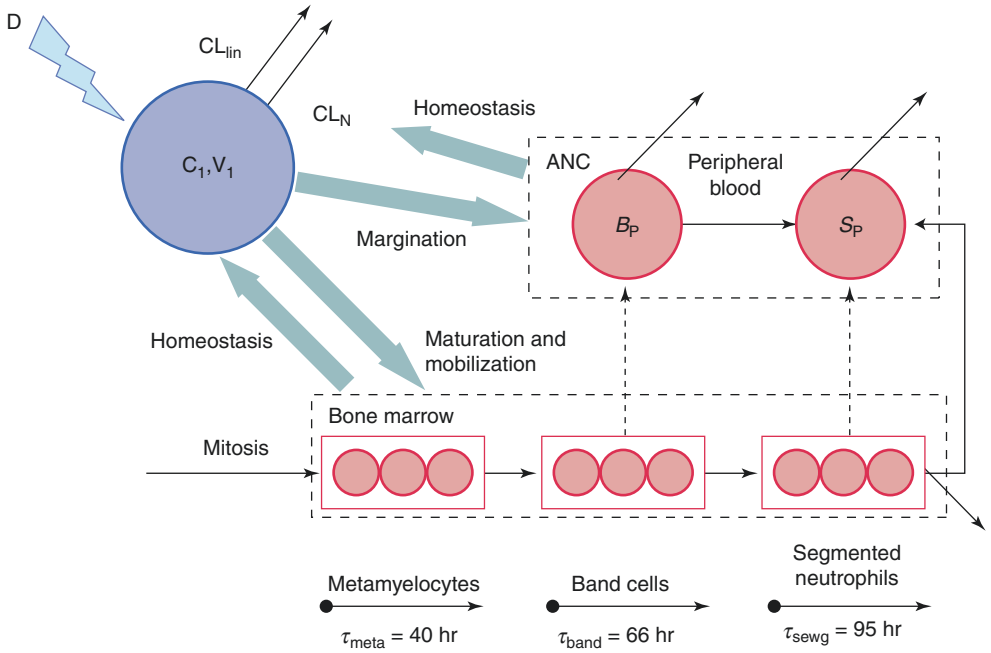


Fig. 6.21 A PK/PD model describing the granulopoietic effects of pegfilgrastim. The PK model is a one-compartment model with two parallel elimination pathways, a first-order elimination process (CL_{lin}), and a neutrophil-mediated elimination process (CL_N). C_1 and V_1 are the concentrations in the PK compartment and the corresponding volume of distribution. The PD model is a cytokinetic model similar to the cell life span model in Fig. 6.19. 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))

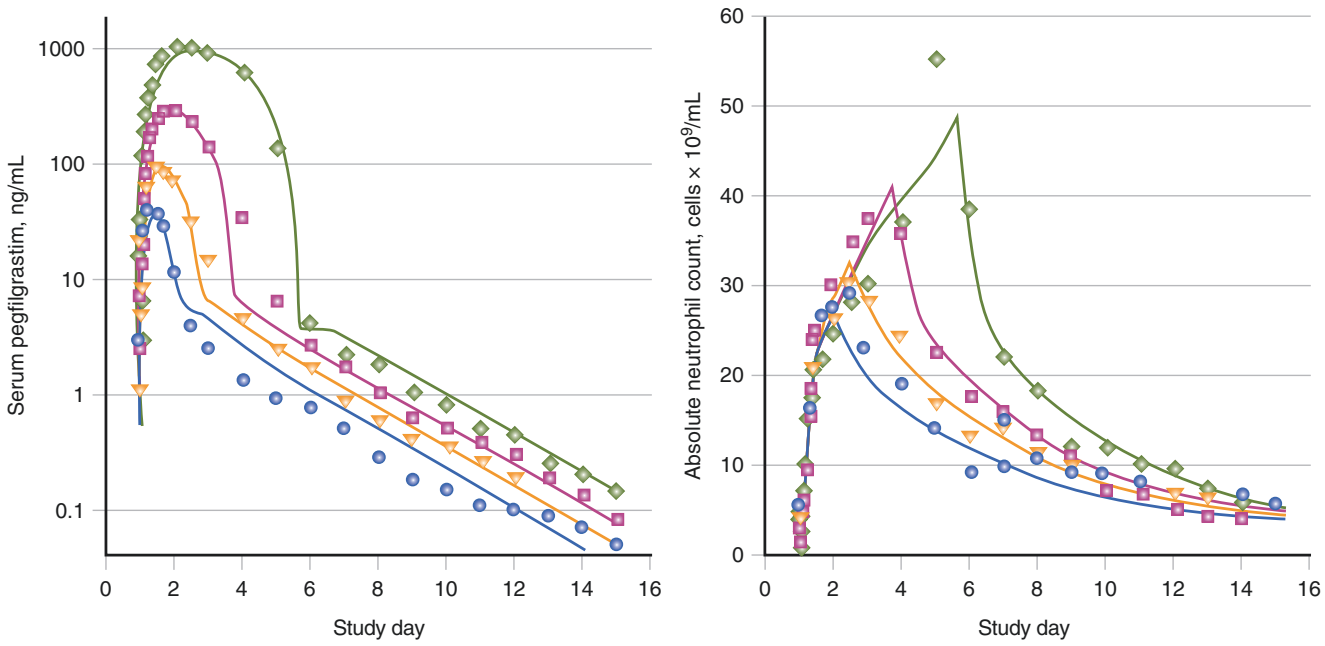


Fig. 6.22 Pegfilgrastim concentration time course and absolute neutrophil count (ANC) time profiles in healthy subjects after a single SC administration of 30, 60, 100, and 300 μg/kg pegfilgrastim ($n = 8$/dose group). Measured data are presented by symbols as mean. Lines represent modeled time courses 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)

Pharmacokinetics of Nucleic Acid-Based Therapeutics

Therapeutic drugs derived from nucleic acids have in the past decade become a rapidly evolving modality in our armamentarium of pharmacologic interventions to treat human disease. In contrast to conventional drugs and therapeutic proteins that generally target proteins in the body, nucleic acid-based therapeutics target in most instances gene expression. Especially four platform technologies have emerged and are increasingly used: (1) chemically modified antisense oligonucleotides (ASOs); (2) N-acetylgalactosamine (GalNAc)-modified short interfering RNA (siRNA) conjugates; (3) lipid nanoparticle (LNP)-based RNA systems; and (4) adeno-associated virus (AAV) vectors (Kulkarni et al. 2021). LNP- and AAV-based systems have recently gained popularity as basis for COVID-19 vaccines and are extensively discussed in Chap. 15. AAV-based technology is also used in approved and investigational gene therapy approaches and is discussed in Chap. 14. Thus, the following section focuses on the pharmacokinetics of ASOs and siRNA.

ASOs are short synthetic single-stranded nucleic acid polymers, usually 15–30 nucleotides in length with a molecular weight of 6–8 kDa that hybridize with cellular RNA using classic Watson-Crick base pairing to modulate gene expression post-transcriptionally. Precise molecular sequence design provides ASOs with high therapeutic potential and specificity compared to other nucleic acid-based drugs. They typically mediate their effects via modulation of pre-RNA splicing, RNA degradation, or regulation of protein translation. In contrast to ASOs, artificial siRNA-based gene regulation relies on sequence complementarity of 7–8 nucleotides of the target mRNA 3′ untranslated region such that a single siRNA may interact with multiple mRNAs with different affinities.

Several classes of chemical modifications were employed over the past decades to reduce the susceptibility of nucleic acid-based drugs to nuclease degradation. First-generation ASOs employed phosphorothioate linkages instead of phosphate linkages in the ASO backbone. The sulfur substitution dramatically modifies the pharmacokinetics of oligonucleotides by stabilizing them against nuclease digestion and increasing nonspecific plasma protein binding, resulting in a prolonged residence time in tissues and cells, improved tissue distribution, and reduced urinary excretion (Geary et al. 2001).

Second generation ASOs further increased nuclease resistance by chemical modification of the ribose close to the 3′- and 5′- end of the molecule, including 2′-O-methoxyethyl (2-MOE) and 2′-fluoro modifications for ASOs and siRNAs. Especially gapmer technology with a central unmodified deoxynucleotide region for optimal RNAse H1 activity and terminal chemical modifications on both, the 3′ and the 5′ ends, has been proven to improve potency and nuclease stability for ASOs (Kulkarni et al. 2021).

siRNAs are large hydrophilic molecules that consist of two complementary strands of RNA that form a double-helical structure of 19–21 base pairs with a molecular mass of ~14 kDa. Their polyanionic backbone and hydrophilic character prevent passive intracellular uptake and therefore require specialized delivery solutions (Migliorati et al. 2022). Similar to ASOs, siRNA also requires chemical modification of the backbone structure to ensure stability in the circulation. To increase target organ accumulation in the liver, siRNA has been conjugated to a triantennary GalNAc moiety that targets the asialoglycoprotein receptor (ASGPR). ASGPR is primarily expressed by hepatocytes and thus allows efficient targeting to this cell population in the liver (Kulkarni et al. 2021).

Pharmacokinetic properties of ASO- and siRNA-based drugs are largely driven by the chemical structure of their backbone and are thus sequence independent within a chemical class. This similarity has been observed in preclinical models as well as in humans. Due to their similar molecular structure, many common class-wide characteristics with regard to pharmacokinetics and drug disposition can be identified across these platform technologies, although each of the currently approved ASO- or siRNA-based therapeutics has unique features (Park et al. 2016; Weidolf et al. 2021).

Administration and Absorption of Nucleic Acid-Based Therapeutics

Similar to proteins, nucleic acids are due to their large molecular size and high molecular charge not orally bioavailable to a relevant degree. Together with their limited stability in the gastrointestinal tract due to nuclease digestion, the resulting oral bioavailability is in the range of 1–3%. Thus, nucleic acid-based therapeutics require parental administration. Most approved ASOs and siRNAs are administered by the IV or SC route. Localized administration by intravitreal or intrathecal administration has shown to be efficacious as well.

Mipomersen, an approved ASO for the treatment of familial hypercholesterolemia, is administered by SC administration. It primarily targets ApoB mRNA in the liver to induce its liver-based degradation. Similarly, inotersen is administered subcutaneously for the treatment of polyneuropathy in patients with hereditary transthyretin-mediated amyloidosis, thereby inhibiting the formation of protein deposits predominantly formed by the liver (Migliorati et al. 2022). Givorisan, inclisiran, and lumasiran are FDA-approved subcutaneously administered GalNAc-conjugated siRNAs for the treatment of acute hepatic porphyria, hypercholesterolemia, and primary hyperoxaluria type 1, respectively (Kulkarni et al.

2021). For nusinersen, an ASO for the treatment of spinal muscle atrophy, circumventing the blood-brain barrier that is usually impenetrable for ASOs, is crucial to achieve its therapeutic target in the brain. This was accomplished by creating a dosage form for intrathecal administration (Luu et al. 2017).

After SC administration of a 2′-MOE gapmer-modified ASO, bioavailability is close to 100% (Geary et al. 2015). Peak plasma concentrations occur 2–4 h after SC administration (Migliorati et al. 2022). For other less metabolically stabilized ASO structures, subcutaneous bioavailability may remain below 40%.

Distribution and Tissue Uptake of Nucleic Acid-Based Therapeutics

ASOs and siRNA generally have multiphasic distribution profiles after intravenous administration, with a rapid initial distribution (3–4 h) and a long terminal half-life that may reach several weeks for second-generation ASOs (Weidolf et al. 2021). As ASOs and siRNA are generally considered to undergo distribution rate-limited elimination, the terminal half-life in tissues is considered to be in parallel with the terminal half-life in plasma (Geary et al. 1997).

After intravenous administration, ASOs are detected in nearly all tissues and organs except for the brain and testes, suggesting significant transport barriers in these tissues. Major accumulation of ASOs occurs in liver and kidneys, and to a lesser extent in spleen, bone marrow, adipose tissue, and lymph nodes, which seems to be independent of ASO sequence. This may, however, limit their viability against diseases with targets in other tissues, such as in the heart or skeletal muscle (Weidolf et al. 2021).

Cellular uptake as prerequisite for distribution and metabolism is predominantly facilitated by clathrin-mediated endocytosis This internalization into endosomes is followed by subcellular endosome release and trafficking. As ASOs have different intracellular target locations (e.g., RNAase H-mediated mRNA degradation in the cytoplasm (ribosomes) or nucleus, vs. exon skipping in the spliceosome in the nucleus), endosomal release and intracellular trafficking are key determinants of the pharmacologic activity of ASO drugs (Juliano 2018).

For siRNA conjugates, clathrin-dependent receptor-mediated endocytosis via ASGPR has been utilized to target and enrich siRNA in hepatocytes. While hepatocytes constitute 80% of the liver volume, only 15% of the amount of unconjugated nucleic acid drugs taken up by the liver reaches hepatocytes, while majority ends up in Kupffer cells and endothelial cells (Wang et al. 2019). Thus, GalNAc conjugation is necessary for efficient drug delivery to hepatocytes.

As previously discussed in this chapter, ASGPR is a promiscuous C-type lectin receptor that recognizes and facilitates the intracellular update of glycans with end-standing galactose or GalNAc. It is primarily expressed on the sinusoidal membrane of hepatocytes and possesses a high internalization and recycling rate for efficient substrate delivery. Thus, it is an ideal conduit to target hepatocytes with ASGPR-recognized glycans, thereby circumventing broad uptake of siRNA to various organs and tissues throughout the body. Thus, siRNA molecules conjugated with triantennary GalNAc sugar moieties achieve substantially higher potency and in vivo efficacy when targeting gene expression mechanisms in hepatocytes (Kulkarni et al. 2021).

Chemical modifications of the phosphorothioate backbone structure have altered protein binding and organ distribution. Chemically modified ASOs and conjugated siRNA are highly bound to plasma proteins, with more than 85% for nusinersen, inotersen, and mipomersen (Crooke and Geary 2013) as well as approved siRNA conjugates (McDougall et al. 2022). This high binding was present in humans as well as mice, rats, and monkeys. Major binding proteins seem to be β_2-macroglobulin and albumin. The high plasma protein binding is a major determinant in the pharmacokinetics of chemically modified ASOs and siRNA. High plasma protein binding, for example, protects ASOs from renal filtration, as based on their molecular weight below the filtration cutoff of the kidneys their unbound fraction undergoes renal filtration. Thus, plasma protein binding severely restricts the renal elimination of ASOs, so that urinary excretion of intact compound is only a minor elimination pathway (Crooke et al. 1996).

Metabolism of Nucleic Acid-Based Therapeutics

Unmodified RNA oligonucleotides are rapidly degraded in biological matrices, thereby limiting their utility as therapeutics. To enable the development of oligonucleotides into viable therapeutic agents, a variety of chemical modifications were necessary to increase their in vivo stability as outlined above. Endonuclease- and exonuclease-mediated degradation occurs in the blood stream and in target cells. For most nucleic acid-based drugs, endonuclease activity occurs first, cleaving the oligonucleotide into fragments. Then, exonuclease activity further degrades the fragments.

While ASOs and siRNA are relatively rapidly removed from blood, predominantly by distribution and uptake into tissues, their residence time in tissues was found to be relatively long and dependent on their chemical modification (Geary et al. 2001). ASOs are cleared from tissues by nuclease-mediated metabolism, with half-lives of up to several weeks (Geary et al. 2015). GalNAc-congugated siRNA metabolism generally results in loss of one, two or all three GalNAc sugar chains, followed by excretion of parts of, or the full triannetary molecule (Weidolf et al. 2021). Knowledge on the metabolizing enzymes is still evolving and may require further investigation.

Plasma concentration–time profiles are predominantly characterized by rapid distribution after intravenous admin-

istration, followed by slow redistribution and tissue elimination in the terminal phase. Thus, terminal half-lives in plasma mirror those in tissues and are driven by slow tissue elimination. The partition ratios between liver and plasma in the post-distribution phase are approximately 5000:1 for 2′MOE-modified ASOs and are species independent. Thus, whole-organ pharmacokinetics after 24 h is thought to present intracellular exposure, as only very little ASO remained bound to extracellular components by 24 h after injection, and post-distribution plasma concentrations can be used as surrogate for tissue exposure in all species, including humans (Geary et al. 2015). Similarly, siRNA levels in hepatocytes are considered better predictors for pharmacologic activity of GalNAc-conjugated siRNA than plasma concentrations (Migliorati et al. 2022).

Givosiran as siRNA conjugate consists of a 21-base sense strand and a 23-base antisense strand that are fully modified with 16 nucleotides containing a 2′-F substitution and the remaining nucleotides being 2′-OMe substituted. Six of its backbone linkages distributed at the ends of the strands are PS-modified. The combination of chemical modification of the backbone and conjugation to tri-GalNAc allows for sufficient metabolic stability and high uptake into the liver for efficient treatment of the approved indication acute hepatic porphyria (Zhang et al. 2021).

So far, little is known on the specific nucleases involved in ASO and siRNA metabolism, such as identity, specificity, capacity, potential competition with endogenous RNA, and interspecies differences. Given the structure and function of RNA are conserved across species, the function and substrate specificity of nucleases are also likely to be conserved, and significant species differences in metabolite structures are not expected (Weidolf et al. 2021).

Excretion of Nucleic Acid-Based Therapeutics

Urinary excretion is a major route of excretion for ASOs, regardless of sequence or chemical structure, with the majority being shorter length metabolites rather than unchanged parent drug (Agrawal et al. 1995). Metabolites are assumed to undergo cellular release via membrane leakage, vesicle release, or endosome release. Only a minor fraction of the dose is excreted into feces although enterohepatic recirculation has been suggested (Dvorchik 2000). For phosphorothioate-modified ASOs, the increased plasma protein binding substantially decreased their renal excretion. For fomivirsen, for example, the excretion of the parent compound was only 16% in urine and 3% in feces. For phosphorothioate ASOs with 2′-MOE gapmer technology, renal excretion of parent drug remains even lower (<1–3%). Metabolite levels remain low in the systemic circulation, but higher percentages are recovered in the urine, likely because of lower plasma protein binding than the parent compound (Weidolf et al. 2021).

Limited assessments of the effects of renal impairment on plasma exposures for ASOs suggest that mild or moderate renal impairment showed no effect on the plasma exposure, but end-stage renal disease may result in a mild increase (34%) (Wang et al. 2023).

Conclusion

The pharmacokinetic and pharmacodynamic characteristics of proteins and nucleic acid-based drugs 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. Rational, scientifically based drug development and pharmacotherapy based on the use of pharmacokinetic and pharmacodynamic concepts will undoubtedly propel the success and future of protein- and nucleic acid-based therapeutics 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?
11. What are common chemical modifications in ASOs and siRNA to increase their stability toward degradation by nucleases?

Answers

1. Proteolysis, glomerular filtration followed by intraluminal metabolism or tubular reabsorption with intracellular lysosomal degradation, and 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. In some cases, protein binding may protect the organism from undesirable, acute effects; in other cases, receptor binding may be facilitated by the binding protein.
4. In some cases, the sugar groups are recognized by hepatic receptors (e.g., mannose by the mannose receptor), facilitating receptor-mediated uptake and metabolism. In other cases, sugar chains and terminal sugar groups (e.g., terminal sialic acid residues) may shield the protein from binding to receptors and hepatic uptake.
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 fluid 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 (modeled with an indirect response or turnover PK/PD model).
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.
11. Substitution of a sulfur atom for a nonbridging oxygen in the phosphate backbone of an oligonucleotide leads to a phosphorothioate linkage between nucleotides that is resistant to nuclease degradation. In addition, select nucleotides are modified at the 2′ position of the ribose, with, for example, a methoxyethyl group (2'-MOE) for ASOs or a fluoride (2'-F) for siRNA.

References

Agoram B, Heatherington AC, Gastonguay MR (2006) Development and evaluation of a population pharmacokinetic-pharmacodynamic model of darbepoetin alfa in patients with nonmyeloid malignancies undergoing multicycle chemotherapy. AAPS J 8(3):E552–E563

Agrawal S, Temsamani J, Galbraith W, Tang J (1995) Pharmacokinetics of antisense oligonucleotides. Clin Pharmacokinet 28(1):7–16

Albitar M, Do KA, Johnson MM et al (2004) Free circulating soluble CD52 as a tumor marker in chronic lymphocytic leukemia and its implication in therapy with anti-CD52 antibodies. Cancer 101(5):999–1008

Allon M, Kleinman K, Walczyk M et al (2002) Pharmacokinetics and pharmacodynamics of darbepoetin alfa and epoetin in patients undergoing dialysis. Clin Pharmacol Ther 72(5):546–555

Anderson PM, Sorenson MA (1994) Effects of route and formulation on clinical pharmacokinetics of interleukin-2. Clin Pharmacokinet 27(1):19–31

Bauer RJ, Gibbons JA, Bell DP, Luo ZP, Young JD (1994) Nonlinear pharmacokinetics of recombinant human macrophage colony- stimulating factor (M-CSF) in rats. J Pharmacol Exp Ther 268(1):152–158

Baxter LT, Zhu H, Mackensen DG, Jain RK (1994) Physiologically based pharmacokinetic model for specific and nonspecific monoclonal antibodies and fragments in normal tissues and human tumor xenografts in nude mice. Cancer Res 54(6):1517–1528

Benincosa LJ, Chow FS, Tobia LP, Kwok DC, Davis CB, Jusko WJ (2000) Pharmacokinetics and pharmacodynamics of a humanized monoclonal antibody to factor IX in cynomolgus monkeys. J Pharmacol Exp Ther 292(2):810–816

Bennett HP, McMartin C (1978) Peptide hormones and their analogues: distribution, clearance from the circulation, and inactivation in vivo. Pharmacol Rev 30(3):247–292

Berdeja J, Jagannath S, Zonder J et al (2016) Pharmacokinetics and safety of Elotuzumab combined with Lenalidomide and dexamethasone in patients with multiple myeloma and various levels of renal impairment: results of a phase Ib study. Clin Lymphoma Myeloma Leuk 16(3):129–138

Boxenbaum H (1982) Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. J Pharmacokinet Biopharm 10(2):201–227

Bressolle F, Audran M, Gareau R, Pham TN, Gomeni R (1997) Comparison of a direct and indirect population pharmacodynamic model: application to recombinant human erythropoietin in athletes. J Pharmacokinet Biopharm 25(3):263–275

Bruin G, Loesche C, Nyirady J, Sander O (2017) Population pharmacokinetic modeling of Secukinumab in patients with moderate to severe psoriasis. J Clin Pharmacol 57(7):876–885

Bu G, Williams S, Strickland DK, Schwartz AL (1992) Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. Proc Natl Acad Sci U S A 89(16):7427–7431

Caliceti P, Veronese FM (2003) Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. Adv Drug Deliv Rev 55(10):1261–1277

Carone FA, Peterson DR (1980) Hydrolysis and transport of small peptides by the proximal tubule. Am J Phys 238(3):F151–F158

Carone FA, Peterson DR, Flouret G (1982) Renal tubular processing of small peptide hormones. J Lab Clin Med 100(1):1–14

Chanson P, Timsit J, Harris AG (1993) Clinical pharmacokinetics of octreotide. Therapeutic applications in patients with pituitary tumours. Clin Pharmacokinet 25(5):375–391

Chiang J, Gloff CA, Yoshizawa CN, Williams GJ (1993) Pharmacokinetics of recombinant human interferon-beta ser in healthy volunteers and its effect on serum neopterin. Pharm Res 10(4):567–572

Chirmule N, Jawa V, Meibohm B (2012) Immunogenicity to therapeutic proteins: impact on PK/PD and efficacy. AAPS J 14(2):296–302

Chow FS, Benincosa LJ, Sheth SB et al (2002) Pharmacokinetic and pharmacodynamic modeling of humanized anti-factor IX antibody (SB 249417) in humans. Clin Pharmacol Ther 71(4):235–245

Colburn W (1991) Peptide, peptoid, and protein pharmacokinetics/pharmacodynamics. In: Garzone P, Colburn W, Mokotoff M (eds) Petides, peptoids, and proteins. Harvey Whitney Books, Cincinnati, OH, pp 94–115

Crooke ST, Geary RS (2013) Clinical pharmacological properties of mipomersen (Kynamro), a second generation antisense inhibitor of apolipoprotein B. Br J Clin Pharmacol 76(2):269–276

Crooke ST, Graham MJ, Zuckerman JE et al (1996) Pharmacokinetic properties of several novel oligonucleotide analogs in mice. J Pharmacol Exp Ther 277(2):923–937

Daniel H, Herget M (1997) Cellular and molecular mechanisms of renal peptide transport. Am J Phys 273(1 Pt 2):F1–F8

Dayneka NL, Garg V, Jusko WJ (1993) Comparison of four basic models of indirect pharmacodynamic responses. J Pharmacokinet Biopharm 21(4):457–478

de la Pena A, Ma X, Reddy S, Ovalle F, Bergenstal RM, Jackson JA (2014) Application of PK/PD modeling and simulation to dosing regimen optimization of high-dose human regular U-500 insulin. J Diabetes Sci Technol 8(4):821–829

Dedrick RL (1973) Animal scale-up. J Pharmacokinet Biopharm 1(5):435–461

Deen WM, Lazzara MJ, Myers BD (2001) Structural determinants of glomerular permeability. Am J Physiol Ren Physiol 281(4):F579–F596

Deng R, Iyer S, Theil FP, Mortensen DL, Fielder PJ, Prabhu S (2011) Projecting human pharmacokinetics of therapeutic antibodies from nonclinical data: what have we learned? MAbs 3(1):61–66

Derendorf H, Meibohm B (1999) Modeling of pharmacokinetic/pharmacodynamic (PK/PD) relationships: concepts and perspectives. Pharm Res 16(2):176–185

Diao L, Meibohm B (2013) Pharmacokinetics and pharmacokinetic-pharmacodynamic correlations of therapeutic peptides. Clin Pharmacokinet 52(10):855–868

Dirks NL, Meibohm B (2010) Population pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet 49(10):633–659

Dirks NL, Nolting A, Kovar A, Meibohm B (2008) Population pharmacokinetics of cetuximab in patients with squamous cell carcinoma of the head and neck. J Clin Pharmacol 48(3):267–278

Dong JQ, Salinger DH, Endres CJ et al (2011) Quantitative prediction of human pharmacokinetics for monoclonal antibodies: retrospective analysis of monkey as a single species for first-in-human prediction. Clin Pharmacokinet 50(2):131–142

Dugger SA, Platt A, Goldstein DB (2018) Drug development in the era of precision medicine. Nat Rev Drug Discov 17(3):183–196

Dvorchik BH (2000) The disposition (ADME) of antisense oligonucleotides. Curr Opin Mol Ther 2(3):253–257

Edwards A, Daniels BS, Deen WM (1999) Ultrastructural model for size selectivity in glomerular filtration. Am J Phys 276(6 Pt 2):F892–F902

EfpiaMidWorkgroup MSF, Burghaus R et al (2016) Good practices in model-informed drug discovery and development: practice, application, and documentation. CPT Pharmacometrics Syst Pharmacol 5(3):93–122

Eigenmann MJ, Fronton L, Grimm HP, Otteneder MB, Krippendorff BF (2017) Quantification of IgG monoclonal antibody clearance in tissues. MAbs 9(6):1007–1015

Eppler SM, Combs DL, Henry TD et al (2002) A target-mediated model to describe the pharmacokinetics and hemodynamic effects of recombinant human vascular endothelial growth factor in humans. Clin Pharmacol Ther 72(1):20–32

Falck D, Thomann M, Lechmann M et al (2021) Glycoform-resolved pharmacokinetic studies in a rat model employing glycoengineered variants of a therapeutic monoclonal antibody. MAbs 13(1):1865596

Fasano A (1998) Novel approaches for oral delivery of macromolecules. J Pharm Sci 87(11):1351–1356

Flessner MF, Lofthouse J, Zakaria el R (1997) In vivo diffusion of immunoglobulin G in muscle: effects of binding, solute exclusion, and lymphatic removal. Am J Phys 273(6 Pt 2):H2783–H2793

Geary RS, Leeds JM, Henry SP, Monteith DK, Levin AA (1997) Antisense oligonucleotide inhibitors for the treatment of cancer: 1. Pharmacokinetic properties of phosphorothioate oligodeoxynucleotides. Anticancer Drug Des 12(5):383–393

Geary RS, Yu RZ, Levin AA (2001) Pharmacokinetics of phosphorothioate antisense oligodeoxynucleotides. Curr Opin Investig Drugs 2(4):562–573

Geary RS, Norris D, Yu R, Bennett CF (2015) Pharmacokinetics, biodistribution and cell uptake of antisense oligonucleotides. Adv Drug Deliv Rev 87:46–51

Gibbs JP, Doshi S, Kuchimanchi M et al (2017) Impact of target-mediated elimination on the dose and regimen of Evolocumab, a human monoclonal antibody against Proprotein convertase Subtilisin/Kexin type 9 (PCSK9). J Clin Pharmacol 57(5):616–626

Glassman PM, Balthasar JP (2016) Physiologically-based pharmacokinetic modeling to predict the clinical pharmacokinetics of monoclonal antibodies. J Pharmacokinet Pharmacodyn 43(4):427–446

Glund S, Gan G, Moschetti V et al (2018) The renal elimination pathways of the dabigatran reversal agent Idarucizumab and its impact on dabigatran elimination. Clin Appl Thromb Hemost 24(5):724–733

Graham ML (2003) Pegaspargase: a review of clinical studies. Adv Drug Deliv Rev 55(10):1293–1302

Handelsman DJ, Swerdloff RS (1986) Pharmacokinetics of gonadotropin-releasing hormone and its analogs. Endocr Rev 7(1):95–105

Hayashida K, Bartlett AH, Chen Y, Park PW (2010) Molecular and cellular mechanisms of ectodomain shedding. Anat Rec (Hoboken) 293(6):925–937

Herbst RS, Langer CJ (2002) Epidermal growth factor receptors as a target for cancer treatment: the emerging role of IMC-C225 in the treatment of lung and head and neck cancers. Semin Oncol 29(1 Suppl 4):27–36

Holford NH, Sheiner LB (1982) Kinetics of pharmacologic response. Pharmacol Ther 16(2):143–166

Inui K, Terada T, Masuda S, Saito H (2000) Physiological and pharmacological implications of peptide transporters, PEPT1 and PEPT2. Nephrol Dial Transplant 15(Suppl 6):11–13

Jin F, Krzyzanski W (2004) Pharmacokinetic model of target-mediated disposition of thrombopoietin. AAPS PharmSci 6(1):E9

Johnson V, Maack T (1977) Renal extraction, filtration, absorption, and catabolism of growth hormone. Am J Phys 233(3):F185–F196

Juliano RL (2018) Intracellular trafficking and endosomal release of oligonucleotides: what we know and what we Don't. Nucleic Acid Ther 28(3):166–177

Kaufman JS, Reda DJ, Fye CL et al (1998) Subcutaneous compared with intravenous epoetin in patients receiving hemodialysis. Department of Veterans Affairs Cooperative Study Group on erythropoietin in hemodialysis patients. N Engl J Med 339(9):578–583

Khor SP, McCarthy K, DuPont M, Murray K, Timony G (2000) Pharmacokinetics, pharmacodynamics, allometry, and dose selection of rPSGL-Ig for phase I trial. J Pharmacol Exp Ther 293(2):618–624

Kim J, Hayton WL, Robinson JM, Anderson CL (2007) Kinetics of FcRn-mediated recycling of IgG and albumin in human: pathophysiology and therapeutic implications using a simplified mechanism-based model. Clin Immunol 122(2):146–155

Kingwell K (2016) Drug delivery: new targets for drug delivery across the BBB. Nat Rev Drug Discov 15(2):84–85

Ko S, Jo M, Jung ST (2021) Recent achievements and challenges in prolonging the serum half-lives of therapeutic IgG antibodies through fc engineering. BioDrugs 35(2):147–157

Kompella U, Lee V (1991) Pharmacokinetics of peptide and protein drugs. In: Lee V (ed) Peptide and protein drug delivery. Marcel Dekker, New York, pp 391–484

Kontermann R (2012) Therapeutic proteins: strategies to modulate their plasma half-lives. Wiley, Weinheim

Krogsgaard Thomsen M, Friis C, Sehested Hansen B et al (1994) Studies on the renal kinetics of growth hormone (GH) and on the GH receptor and related effects in animals. J Pediatr Endocrinol 7(2):93–105

Kulkarni JA, Witzigmann D, Thomson SB et al (2021) The current landscape of nucleic acid therapeutics. Nat Nanotechnol 16(6):630–643

Kuwabara T, Uchimura T, Kobayashi H, Kobayashi S, Sugiyama Y (1995) Receptor-mediated clearance of G-CSF derivative nartograstim in bone marrow of rats. Am J Phys 269(1 Pt 1):E1–E9

Lee HJ (2002) Protein drug oral delivery: the recent progress. Arch Pharm Res 25(5):572–584

Lee H, Kimko HC, Rogge M, Wang D, Nestorov I, Peck CC (2003) Population pharmacokinetic and pharmacodynamic modeling of etanercept using logistic regression analysis. Clin Pharmacol Ther 73(4):348–365

Lesko LJ (2007) Paving the critical path: how can clinical pharmacology help achieve the vision? Clin Pharmacol Ther 81(2):170–177

Lesko LJ, Rowland M, Peck CC, Blaschke TF (2000) Optimizing the science of drug development: opportunities for better candidate selection and accelerated evaluation in humans. J Clin Pharmacol 40(8):803–814

Levy G (1986) Kinetics of drug action: an overview. J Allergy Clin Immunol 78(4 Pt 2):754–761

Levy G (1994) Mechanism-based pharmacodynamic modeling. Clin Pharmacol Ther 56(4):356–358

Limothai W, Meibohm B (2011) Effect of dose on the apparent bioavailability of therapeutic proteins that undergo target-mediated drug disposition. AAPS J 13(S2)

Liu Q, Ahadpour M, Rocca M, Huang SM (2021) Clinical pharmacology regulatory sciences in drug development and precision medicine: current status and emerging trends. AAPS J 23(3):54

Lunghi B, Morfini M, Martinelli N et al (2022) The Asialoglycoprotein receptor minor subunit gene contributes to pharmacokinetics of factor VIII concentrates in hemophilia a. Thromb Haemost 122(5):715–725

Luu KT, Norris DA, Gunawan R, Henry S, Geary R, Wang Y (2017) Population pharmacokinetics of Nusinersen in the cerebral spinal fluid and plasma of pediatric patients with spinal muscular atrophy following intrathecal administrations. J Clin Pharmacol 57(8):1031–1041

Maack T, Park C, Camargo M (1985) Renal filtration, transport and metabolism of proteins. In: Seldin D, Giebisch G (eds) The kidney. Raven Press, New York, pp 1773–1803

Mach H, Gregory SM, Mackiewicz A et al (2011) Electrostatic interactions of monoclonal antibodies with subcutaneous tissue. Ther Deliv 2(6):727–736

Mager DE (2006) Target-mediated drug disposition and dynamics. Biochem Pharmacol 72(1):1–10

Mager DE, Wyska E, Jusko WJ (2003) Diversity of mechanism-based pharmacodynamic models. Drug Metab Dispos 31(5):510–518

Mahato RI, Narang AS, Thoma L, Miller DD (2003) Emerging trends in oral delivery of peptide and protein drugs. Crit Rev Ther Drug Carrier Syst 20(2–3):153–214

McDougall R, Ramsden D, Agarwal S et al (2022) The nonclinical disposition and pharmacokinetic/Pharmacodynamic properties of N-Acetylgalactosamine-conjugated small interfering RNA are highly predictable and build confidence in translation to human. Drug Metab Dispos 50(6):781–797

McMahon HT, Boucrot E (2011) Molecular mechanism and physiological functions of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 12(8):517–533

McMartin C (1992) Pharmacokinetics of peptides and proteins: opportunities and challenges. Adv Drug Res 22:39–106

Meibohm B (2004) Pharmacokinetics of protein- and nucleotide-based drugs. In: Mahato RI (ed) Biomaterials for delivery and targeting of proteins and nucleic acids. CRC Press, Boca Raton, pp 275–294

Meibohm B (2006) Pharmacokinetics and pharmacodynamics of biotech drugs. Wiley, Weinheim

Meibohm B, Derendorf H (1997) Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. Int J Clin Pharmacol Ther 35(10):401–413

Meibohm B, Derendorf H (2002) Pharmacokinetic/pharmacodynamic studies in drug product development. J Pharm Sci 91(1):18–31

Meibohm B, Zhou H (2012) Characterizing the impact of renal impairment on the clinical pharmacology of biologics. J Clin Pharmacol 52(1 Suppl):54S–62S

Meijer D, Ziegler K (1993) Biological barriers to protein delivery. Plenum Press, New York

Migliorati JM, Liu S, Liu A et al (2022) Absorption, distribution, metabolism, and excretion of US Food and Drug Administration-approved antisense oligonucleotide drugs. Drug Metab Dispos 50(6):888–897

Mohler M, Cook J, Lewis D et al (1993) Altered pharmacokinetics of recombinant human deoxyribonuclease in rats due to the presence of a binding protein. Drug Metab Dispos 21(1):71–75

Molineux G (2003) Pegylation: engineering improved biopharmaceuticals for oncology. Pharmacotherapy 23(8 Pt 2):3S–8S

Montero-Julian FA, Klein B, Gautherot E, Brailly H (1995) Pharmacokinetic study of anti-interleukin-6 (IL-6) therapy with monoclonal antibodies: enhancement of IL-6 clearance by cocktails of anti-IL-6 antibodies. Blood 85(4):917–924

Mordenti J, Chen SA, Moore JA, Ferraiolo BL, Green JD (1991) Interspecies scaling of clearance and volume of distribution data for five therapeutic proteins. Pharm Res 8(11):1351–1359

Mould DR, Meibohm B (2016) Drug development of therapeutic monoclonal antibodies. BioDrugs 30(4):275–293

Mould DR, Davis CB, Minthorn EA et al (1999) A population pharmacokinetic-pharmacodynamic analysis of single doses of clenoliximab in patients with rheumatoid arthritis. Clin Pharmacol Ther 66(3):246–257

Nielsen S, Nielsen JT, Christensen EI (1987) Luminal and basolateral uptake of insulin in isolated, perfused, proximal tubules. Am J Phys 253(5 Pt 2):F857–F867

Nolain P, Djebli N, Brunet A, Fabre D, Khier S (2022) Combined semi-mechanistic target-mediated drug disposition and pharmacokinetic-Pharmacodynamic models of Alirocumab, PCSK9, and low-density lipoprotein cholesterol in a pooled analysis of randomized phase I/II/III studies. Eur J Drug Metab Pharmacokinet 47(6):789–802

Ober RJ, Radu CG, Ghetie V, Ward ES (2001) Differences in promiscuity for antibody-FcRn interactions across species: implications for therapeutic antibodies. Int Immunol 13(12):1551–1559

Park J, Park J, Pei Y, Xu J, Yeo Y (2016) Pharmacokinetics and biodistribution of recently-developed siRNA nanomedicines. Adv Drug Deliv Rev 104:93–109

Pauletti GM, Gangwar S, Siahaan TJ, Jeffrey A, Borchardt RT (1997) Improvement of oral peptide bioavailability: Peptidomimetics and prodrug strategies. Adv Drug Deliv Rev 27(2–3):235–256

Peck CC, Barr WH, Benet LZ et al (1994) Opportunities for integration of pharmacokinetics, pharmacodynamics, and toxicokinetics in rational drug development. J Clin Pharmacol 34(2):111–119

Perez-Ruixo JJ, Kimko HC, Chow AT, Piotrovsky V, Krzyzanski W, Jusko WJ (2005) Population cell life span models for effects of drugs following indirect mechanisms of action. J Pharmacokinet Pharmacodyn 32(5–6):767–793

Periti P, Mazzei T, Mini E (2002) Clinical pharmacokinetics of depot leuprorelin. Clin Pharmacokinet 41(7):485–504

Perrier D, Mayersohn M (1982) Noncompartmental determination of the steady-state volume of distribution for any mode of administration. J Pharm Sci 71(3):372–373

Piscitelli SC, Reiss WG, Figg WD, Petros WP (1997) Pharmacokinetic studies with recombinant cytokines. Scientific issues and practical considerations. Clin Pharmacokinet 32(5):368–381

Porter CJ, Charman SA (2000) Lymphatic transport of proteins after subcutaneous administration. J Pharm Sci 89(3):297–310

Rabkin R, Ryan MP, Duckworth WC (1984) The renal metabolism of insulin. Diabetologia 27(3):351–357

Racine-Poon A, Botta L, Chang TW et al (1997) Efficacy, pharmacodynamics, and pharmacokinetics of CGP 51901, an anti- immunoglobulin E chimeric monoclonal antibody, in patients with seasonal allergic rhinitis. Clin Pharmacol Ther 62(6):675–690

Radwanski E, Chakraborty A, Van Wart S et al (1998) Pharmacokinetics and leukocyte responses of recombinant human interleukin-10. Pharm Res 15(12):1895–1901

Ramakrishnan R, Cheung WK, Wacholtz MC, Minton N, Jusko WJ (2004) Pharmacokinetic and pharmacodynamic modeling of recombinant human erythropoietin after single and multiple doses in healthy volunteers. J Clin Pharmacol 44(9):991–1002

Reddy ST, Berk DA, Jain RK, Swartz MA (2006) A sensitive in vivo model for quantifying interstitial convective transport of injected macromolecules and nanoparticles. J Appl Physiol 101(4):1162–1169

Richter WF, Gallati H, Schiller CD (1999) Animal pharmacokinetics of the tumor necrosis factor receptor-immunoglobulin fusion protein lenercept and their extrapolation to humans. Drug Metab Dispos 27(1):21–25

Richter WF, Bhansali SG, Morris ME (2012) Mechanistic determinants of biotherapeutics absorption following SC administration. AAPS J 14(3):559–570

Roopenian DC, Akilesh S (2007) FcRn: the neonatal fc receptor comes of age. Nat Rev Immunol 7(9):715–725

Roskos LK, Lum P, Lockbaum P, Schwab G, Yang BB (2006) Pharmacokinetic/pharmacodynamic modeling of pegfilgrastim in healthy subjects. J Clin Pharmacol 46(7):747–757

Ryman JT, Meibohm B (2017) Pharmacokinetics of monoclonal antibodies. CPT Pharmacometrics Syst Pharmacol 6(9):576–588

Schomburg A, Kirchner H, Atzpodien J (1993) Renal, metabolic, and hemodynamic side-effects of interleukin-2 and/or interferon alpha: evidence of a risk/benefit advantage of subcutaneous therapy. J Cancer Res Clin Oncol 119(12):745–755

Sharma A, Jusko W (1998) Characteristics of indirect pharmacodynamic models and applications to clinical drug responses. Br J Clin Pharmacol 45:229–239

Sheiner LB, Steimer JL (2000) Pharmacokinetic/pharmacodynamic modeling in drug development. Annu Rev Pharmacol Toxicol 40:67–95

Sheiner LB, Stanski DR, Vozeh S, Miller RD, Ham J (1979) Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. Clin Pharmacol Ther 25(3):358–371

Shen WC (2003) Oral peptide and protein delivery: unfulfilled promises? Drug Discov Today 8(14):607–608

Smedsrod B, Einarsson M (1990) Clearance of tissue plasminogen activator by mannose and galactose receptors in the liver. Thromb Haemost 63(1):60–66

Straughn AB (1982) Model-independent steady-state volume of distribution. J Pharm Sci 71(5):597–598

Straughn AB (2006) Limitations of noncompartmental pharmacokinetic analysis of biotech drugs. In: Meibohm B (ed) Pharmacokinetics and pharmacodynamics of biotech drugs. Wiley, Weinheim, pp 181–188

Strickland DK, Kounnas MZ, Argraves WS (1995) LDL receptor-related protein: a multiligand receptor for lipoprotein and proteinase catabolism. FASEB J 9(10):890–898
Sun YN, Jusko WJ (1999) Role of baseline parameters in determining indirect pharmacodynamic responses. J Pharm Sci 88(10):987–990
Sun YN, Lee HJ, Almon RR, Jusko WJ (1999) A pharmacokinetic/pharmacodynamic model for recombinant human growth hormone effects on induction of insulin-like growth factor I in monkeys. J Pharmacol Exp Ther 289(3):1523–1532
Supersaxo A, Hein W, Gallati H, Steffen H (1988) Recombinant human interferon alpha-2a: delivery to lymphoid tissue by selected modes of application. Pharm Res 5(8):472–476
Supersaxo A, Hein WR, Steffen H (1990) Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcutaneous administration. Pharm Res 7(2):167–169
Suryawanshi S, Zhang L, Pfister M, Meibohm B (2010) The current role of model-based drug development. Expert Opin Drug Discovery 5(4):311–321
Tabrizi M, Roskos LK (2006) Exposure-Reponse relationships for therapeutic biologics. In: Meibohm B (ed) Pharmacokinetics and pharmacodynamics of biotech drugs. Wiley, Weinheim, pp 295–330
Takagi A, Masuda H, Takakura Y, Hashida M (1995) Disposition characteristics of recombinant human interleukin-11 after a bolus intravenous administration in mice. J Pharmacol Exp Ther 275(2):537–543
Taki Y, Sakane T, Nadai T et al (1998) First-pass metabolism of peptide drugs in rat perfused liver. J Pharm Pharmacol 50(9):1013–1018
Tang L, Meibohm B (2006) Pharmacokinetics of peptides and proteins. In: Meibohm B (ed) Pharmacokinetics and pharmacodynamics of biotech drugs. Wiley, Weinheim, pp 17–44
Tang L, Persky AM, Hochhaus G, Meibohm B (2004) Pharmacokinetic aspects of biotechnology products. J Pharm Sci 93(9):2184–2204
Tanswell P, Modi N, Combs D, Danays T (2002) Pharmacokinetics and pharmacodynamics of tenecteplase in fibrinolytic therapy of acute myocardial infarction. Clin Pharmacokinet 41(15):1229–1245
Toon S (1996) The relevance of pharmacokinetics in the development of biotechnology products. Eur J Drug Metab Pharmacokinet 21(2):93–103
Veng-Pedersen P, Gillespie W (1984) Mean residence time in peripheral tissue: a linear disposition parameter useful for evaluating a drug's tissue distribution. J Pharmacokinet Biopharm 12(5):535–543
Verma S, Goand UK, Husain A, Katekar RA, Garg R, Gayen JR (2021) Challenges of peptide and protein drug delivery by oral route: current strategies to improve the bioavailability. Drug Dev Res 82(7):927–944
Veronese FM, Caliceti P (2006) Custom-tailored pharmacokinetics and pharmacodynamics via chemical modifications of biotech drugs. In: Meibohm B (ed) Pharmacokinetics and pharmacodynamics of Boptech drugs. Wiley, Weinheim, pp 271–294
Walsh S, Shah A, Mond J (2003) Improved pharmacokinetics and reduced antibody reactivity of lysostaphin conjugated to polyethylene glycol. Antimicrob Agents Chemother 47(2):554–558
Wang W, Wang EQ, Balthasar JP (2008) Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 84(5):548–558
Wang Y, Yu RZ, Henry S, Geary RS (2019) Pharmacokinetics and clinical pharmacology considerations of GalNAc(3)-conjugated antisense oligonucleotides. Expert Opin Drug Metab Toxicol 15(6):475–485
Wang Y, Diep JK, Yu RZ et al (2023) Assessment of the effect of organ impairment on the pharmacokinetics of 2'-MOE and Phosphorothioate modified antisense oligonucleotides. J Clin Pharmacol 63(1):21–28
Weidolf L, Bjorkbom A, Dahlen A et al (2021) Distribution and biotransformation of therapeutic antisense oligonucleotides and conjugates. Drug Discov Today 26(10):2244–2258
Wills RJ, Ferraiolo BL (1992) The role of pharmacokinetics in the development of biotechnologically derived agents. Clin Pharmacokinet 23(6):406–414
Yang BB (2006) Integration of pharmacokinetics and pharmacodynamics into the drug developmetn of pegfilgrastim, a pegylated protein. In: Meibohm B (ed) Pharmacokinetics and pharmacodynamics of biotech drugs. Wiley, Weinheim, pp 373–394
Yapa SW, Roth D, Gordon D, Struemper H (2016) Comparison of intravenous and subcutaneous exposure supporting dose selection of subcutaneous belimumab systemic lupus erythematosus phase 3 program. Lupus 25(13):1448–1455
Zamboni WC (2003) Pharmacokinetics of pegfilgrastim. Pharmacotherapy 23(8 Pt 2):9S–14S
Zhang Y, Meibohm B (2012) Pharmacokinetics and pharmacodynamics and therapeutic peptides and proteins. In: Kayzer O, Warzecha H (eds) Pharmaceutical biotechnology: drug discovery and clinical Apllications. Wiley, Weinheim, pp 337–368
Zhang L, Pfister M, Meibohm B (2008) Concepts and challenges in quantitative pharmacology and model-based drug development. AAPS J 10(4):552–559
Zhang MM, Bahal R, Rasmussen TP, Manautou JE, Zhong XB (2021) The growth of siRNA-based therapeutics: updated clinical studies. Biochem Pharmacol 189:114432
Zhao L, Ji P, Li Z, Roy P, Sahajwalla CG (2013) The antibody drug absorption following subcutaneous or intramuscular administration and its mathematical description by coupling physiologically based absorption process with the conventional compartment pharmacokinetic model. J Clin Pharmacol 53(3):314–325
Zhu H, Huang SM, Madabushi R, Strauss DG, Wang Y, Zineh I (2019) Model-informed drug development: a regulatory perspective on Progress. Clin Pharmacol Ther 106(1):91–93
Zia-Amirhosseini P, Minthorn E, Benincosa LJ et al (1999) Pharmacokinetics and pharmacodynamics of SB-240563, a humanized monoclonal antibody directed to human interleukin-5, in monkeys. J Pharmacol Exp Ther 291(3):1060–1067
Zito SW (1997) Pharmaceutical biotechnology: a programmed text, vol 10. Technomic Pub. Co., Lancaster, PA, p 328

Further Reading

General Pharmacokinetics and Pharmacodynamics

Bonate PL (2011) Pharmacokinetic-pharmacodynamic modeling and simulation. Springer, New York
Derendorf H, Meibohm B (1999) Modeling of pharmacokinetic/pharmacodynamic (PK/PD) relationships: concepts and perspectives. Pharm Res 16(2):176–185
Derendorf H, Schmidt S (2019) Rowland and Tozer's clinical pharmacokinetics and pharmacodynamics: concepts and applications. Lippincott, Baltimore
Gabrielsson J, Hjorth S (2012) Quantitative pharmacology. Swedish Academy of Pharmaceutical Sciences, Stockholm
Gibaldi M, Perrier D (1982) Pharmacokinetics. Marcel Dekker Inc., New York
Holford NH, Sheiner LB (1982) Kinetics of pharmacologic response. Pharmacol Ther 16(2):143–166
Huang SM, Lertora JJ, Atkinson AJ (2012) Principles of clinical pharmacology. Academic, San Diego

Pharmacokinetics and Pharmacodynamics of Proteins

Diao L, Meibohm B (2013) Pharmacokinetics and pharmacokinetic-pharmacodynamic correlations of therapeutic peptides. Clin Pharmacokinet 52(10):855–868

Diao L, Meibohm B (2015) Tools for predicting the PK/PD of therapeutic proteins. Expert Opin Drug Metab Toxicol 11(7):1115–1125

Kontermann R (2012) Therapeutic proteins: strategies to modulate their plasma half-lives. Wiley, Weinheim

Meibohm B (2006) Pharmacokinetics and pharmacodynamics of biotech drugs. Wiley, Weinheim

Mould DR, Meibohm B (2016) Drug development of therapeutic monoclonal antibodies. BioDrugs 30(4):275–293

Ryman JT, Meibohm B (2017) Pharmacokinetics of monoclonal antibodies. CPT Pharmacometrics Syst Pharmacol 6(9):576–588

Tang L, Persky AM, Hochhaus G, Meibohm B (2004) Pharmacokinetic aspects of biotechnology products. J Pharm Sci 93(9):2184–2204

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Weidolf L et al (2021) Distribution and biotransformation of therapeutic antisense oligonucleotides and conjugates. Drug Discov Today 26(10):2244–2258