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Expert Review

Physiologically-Based PK/PD Modelling of Therapeutic Macromolecules

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Abstract. Therapeutic proteins are a diverse class of drugs consisting of naturally occurring or modified proteins, and due to their size and physico-chemical properties, they can pose challenges for the pharmacokinetic and pharmacodynamic studies. Physiologically-based pharmacokinetics (PBPK) modelling has been effective for early in silico prediction of pharmacokinetic properties of new drugs. The aim of the present workshop was to discuss the feasibility of PBPK modelling of macromolecules. The classical PBPK approach was discussed with a presentation of the successful example of PBPK modelling of cyclosporine A. PBPK model was performed with transport of the cyclosporine across cell membranes, affinity to plasma proteins and active membrane transporters included to describe drug transport between physiological compartments. For macromolecules, complex PBPK modelling or permeability-limited and/or target-mediated distribution was discussed. It was generally agreed that PBPK modelling was feasible and desirable. The role of the lymphatic system should be considered when absorption after extravascular administration is modelled. Target-mediated drug disposition was regarded as an important feature for generation of PK models. Complex PK-models may not be necessary when a limited number of organs are affected. More mechanistic PK/PD models will be relevant when adverse events/toxicity are included in the PK/PD modelling.

KEY WORDS: convective distribution; cyclosporin A; erythropoietin; interspecies scaling; macromolecules; monoclonal antibodies; natural cell lifespan concept; neonatal Fc receptors; non-linear pharmacokinetics; permeability-limited distribution; physiologically-based pharmacokinetic modelling; PK/PD modelling; target-mediated drug disposition.

INTRODUCTION

Therapeutic macromolecules are biotechnology-derived products that are mostly used for diagnosis, prevention and treatment of serious and chronic diseases. Many of these molecules are endogenous compounds that are produced by modern biotechnology in quantities that enable the use of both pharmacological and physiological amounts in disease treatment. In recent years, the therapeutic macromolecules have been a major focus of research and development in academia and the pharmaceutical industry (1). This also includes pharmacokinetic and pharmacodynamic principles as knowledge of dose-response and/or concentration-effect relationships are crucial to the development, because it lays the ground-work for rational design of dosing regimen and clinical application.

Therapeutic macromolecules are a chemically diverse group of molecules consisting of proteins, polysaccharides and oligonucleotide gene therapy products. Although gene therapy is an emerging technology with a significant clinical potential, only limited interest in the pharmacokinetic and pharmacodynamic aspects has been shown. A few publications describing the pharmacokinetic and pharmacodymanic considerations in gene therapy have been described (2). This review will only focus on the therapeutic proteins, as they are currently the largest and most advanced group of therapeutic macromolecules.

Meeting Report from an expert meeting organised by COST Action B25. The workshop entitled "Physiologically-based PK/PD modelling of therapeutic macromolecules" was held in Athens, 11 December 2006. COST is the acronym for European Cooperation in the Field of Scientific and Technical Research. COST Action B25 was launched in 2005 and is entitled "Physiologically based pharmaco-/toxicokinetics and dynamics." Invited speakers gave presentations on various aspects of physiologically-based PK/PD modelling. Members of the COST Action B25, Working group 1 were Achiel Van Peer (Belgium), Panos Macheras (Greece), Peter Thygesen (Denmark), Constantin Mircioiu (Romania), Melih Babaoglu (Turkey), Jose A. Guimares Morais (Portugal), Jean-Louis Steimer (Switzerland). The invited experts were Stefan Willmann (Bayer Technology Services, Germany), Kim Kristensen (AstraZeneca, Sweden), Ryossei Kawai (Novartis, Japan), Phil Lowe (Novartis, Switzerland), Bill Jusko (University of Buffalo, USA) and Rune Overgaard (Novo Nordisk, Denmark). Lene Alifrangis (Novo Nordisk, Denmark) participated as an observer. The aims of the workshop were i) to discuss the feasibility of physiologically-based PK/PD modelling of therapeutic macromolecules, and ii) to identify important modelling issues with respect to therapeutic macromolecules.

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Therapeutic proteins are a diverse and complex class of drugs. They often consist of naturally occurring protein substances in the organism, and due to their size and physico-chemical properties, like protein folding, formulation and lack of long-term stability, they can pose many challenges for the pharmacokinetic and pharmacodynamic studies. Treatment with exogenous proteins can affect the stimulation or feedback mechanism in the body and thereby complicate the pharmacokinetic parameter estimation. For many therapeutic macromolecules, the immunoassays and bioassays are often less precise than conventional assays and lead to an assay-dependent pharmacokinetic parameter estimation. Changes in chemical structure or formulation may cause changes in the pharmacokinetics of these compounds. The molecular size of these drugs may affect the absorption after extravascular administration. The clearance of therapeutic macromolecules often involves several mechanisms, like receptor-mediated or immune-mediated reactions, that result in non-linear clearance. Some therapeutic macromolecules are species-specific in pharmacodynamic activity, and, therefore, clearance mechanisms may vary from species to species (3).

PBPK modelling has been demonstrated to be an effective tool for early in silico prediction of pharmacokinetic properties of new drugs. It has gained wide spread use as an attractive alternative to the more empirical compartmental models used in pharmacokinetics because it provides a mechanistic and more realistic approach to describe the behaviour of drugs and may answer questions such as: 1) Why do we observe such behaviour? 2) Can we explain differences among compounds? 3) Can we predict events occurring with drugs at target and other sites, with respect to age, and co-administered drugs (4).

It is the aim of this paper to discuss the feasibility of PBPK and PBPK/PD modelling of therapeutic macromolecules and compare it with that of small molecules in terms of usability for optimal dose regimen design. In addition, important modelling issues with respect to therapeutic macromolecules that may be of special importance during the drug development will be discussed.

BACKGROUND OF PHYSIOLOGICALLY-BASED APPROACHES

Principles of Physiologically-Based Pharmacokinetic Modelling

A major cause of failure of potential drug candidates in the development process is poor pharmacokinetic properties. Until recently, up to 50% of drug development projects failed due to poor ADME properties (absorption, distribution, metabolism and excretion), emphasising the importance of early assessment of these properties in drug development (1).

Traditionally, pharmacokinetic assessment and modelling have been based on empirical models, such as compartmental models or sums of exponentials, where the complexity of the structural model was defined by the experimental data. These models were used to describe the experimental data rather than trying to explain them. PBPK modelling is an important tool for early in silico prediction of pharmacokinetic properties. PBPK models integrate drug-specific data, like intrinsic clearance and tissue-plasma partition coefficients, with an essential drug-independent structural model consisting of organs and tissues of the body combined via the vascular system. Because the structural model is relatively common to most mammalian species, the PBPK model can be used for interspecies scaling. A PBPK model can describe the drug disposition in both blood and various organs and tissues, including those where drug action or elimination occurs. A PBPK model improves the ability to relate pharmacokinetic performance to physiological and physico-chemical properties. The PBPK model also predicts variation in drug concentration in particular organs or tissues of interest, as a function of whole body pharmacokinetics. Consequently, PBPK models are highly complex in nature and give a mechanistic account for the experimental data (4).

When using PBPK models, it is very important to distinguish between models for data analysis and models for simulation. In the data analysis, the crucial element is parameter estimation, whereas simulation models are typically used for evaluating different dosing regimens and scenarios, including extreme situations with overdosing and toxic exposure, by answering the so called “what if” questions. PBPK models enable the prediction of concentrations in various parts of the body and could potentially, on a mechanistically basis, indicate where unwanted reactions would be expected. It is also possible to simulate the impact of changes in pathological conditions (e.g. kidney and liver functions) on the drug concentrations in the body and thereby identify potential risk groups in the general patient population (5).

Pharmacodynamic components, mechanistic or otherwise, may also be included, making the model a combined PBPK/PD model, a powerful tool in drug development, that through preclinical and clinical studies may lead to earlier identification of optimal dosing regimen in clinical trial and shortening the overall development time and costs (4).

From Organ to Whole Body: The Generic PBPK Models

The concept of physiologically-based PK/PD modelling has been described in several papers during the last decades. The generic whole-body PBPK model is typically modelling the organism as a closed circulatory system with a range of interconnecting compartments each representing an organ or specific tissue. The transport between the organs and tissues is described by mass balance equations, where organ- and tissue-specific blood flows are defining the input into and output from the compartments. The arterial and venous blood connects most organs, whereas the flow from the GI tract, spleen and pancreas goes via the portal vein to the liver before it reaches the venous side. The circle is closed by the pulmonary flow. In order to keep the mass balance, the sum of the non-portal venous flows must equal the blood flow through the lung, and the portal flow must equal the arterial flow from the GI tract, spleen and pancreas (4–6). A schematic whole-body PBPK model is shown in Fig. 1.

Usually, in subsections of the whole-body PBPK model relevant for the test compounds, absorption, distribution, metabolism or excretion are described in more detail. In the relevant organs or tissues, it can be appropriate to distinguish between blood, interstitial and intracellular space because these compartments are separated from plasma by membranes that can form physiological barriers. For compounds

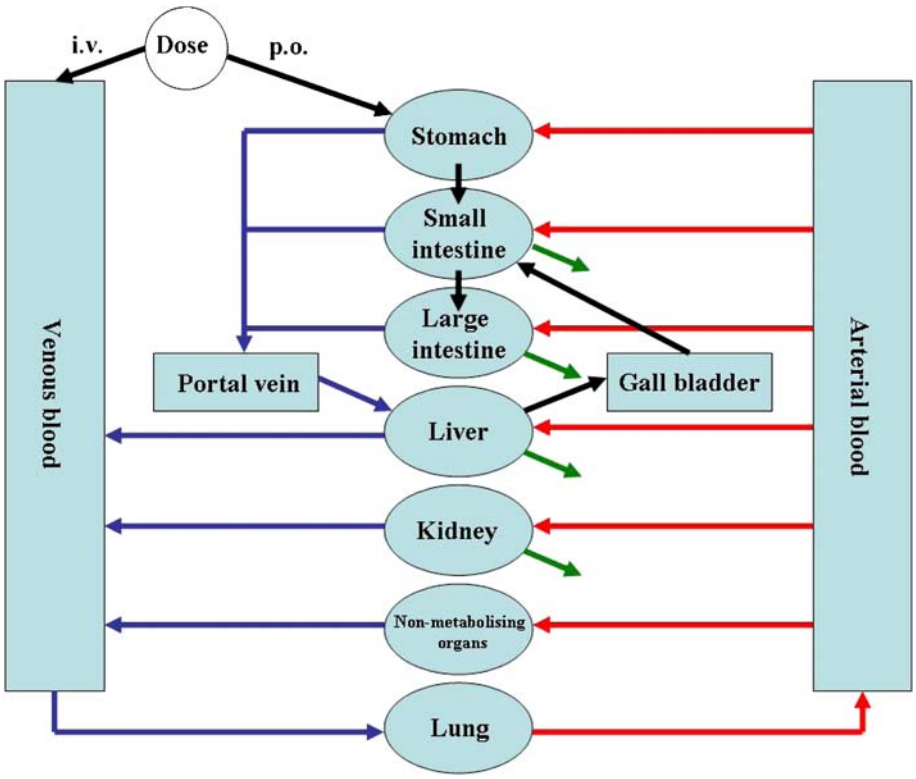

Fig. 1. A typical whole-body PBPK model. The various organs and tissues are organised in a realistic anatomical and physiological order and connected via the circulation.

with permeability-limited kinetics, the diffusion across these physiological barriers is the rate-limiting step for distribution (5,7,8). The interstitial space is separated from plasma by the endothelial membrane. In most organs, a rapid diffusion and equilibrium between plasma and interstitial fluid occurs because of the fenestral structures of the endothelium. This is especially true for the small molecules. For larger molecules, such as antibodies and other macromolecules, the diffusion across the endothelium is very important for the regulation of the tissue distribution. For transport, between the interstitial space and the intercellular space the cell membrane is the diffusion barrier. The diffusion across the cell membrane has been described in several papers for both small and large molecules (5,7,9,10). The diffusion depends on a compound-specific permeability (P) and a tissue-specific surface area (SA), P x SA. The permeability is determined by the diffusion coefficient, a measure of the rate of entry into the cytoplasm depending on the molecular weight or size of a molecule, the partition coefficient and the cell membrane thickness. The partition coefficient for a compound is dependent on the physiochemical properties, such as lipophilicity and acid/base properties, where highly lipophilic and uncharged compounds will have a high partition coefficient and a high permeability and will readily diffuse across the cell membrane (5,11,12). Macromolecules, however, are characterised by limited passage across cell membranes due to their size and charge. Transport across the cell membrane is almost entirely in the form of carrier-mediated transmembrane influx and efflux combined with binding to intravascular or extravascular proteins. Besides the physiochemical properties and protein binding, site-specific and target-oriented receptor-mediated uptake may also be very important for the distribution and elimination of macromolecules (5,13). Fig. 2 describes the mechanisms involved at the distribution at the suborgan level.

For organs and tissues with little impact on the pharmacokinetics of the test compound, a simplified approach using the principles of the well-stirred model and blood flow can be taken. Alternatively, compartmental lumping may be used to reduce the number of compartments and parameters required in the PBPK model. PBPK model lumping is highly compound-dependent and requires a prior knowledge of the pharmacokinetic properties of the test compound (5,8,14,15). Lumping also depends on the intended use of the PBPK model. In cases of restricted distribution and elimination, lumping can be applied to organs not involved in these processes. This is also the case for a well-defined target organ if the model is used to support a PK/PD model. However, in situations where the PBPK model is used for extrapolation between patient subpopulations or for interspecies scaling, the full model with real physiological data is required so the extrapolation will be mechanism-based.

PHYSIOLOGICALLY-BASED MODELLING AND PREDICTION FOR SMALL MOLECULES

Modelling Individuals *Versus* Populations

The use of PBPK modelling has been demonstrated in a number of publications within the last two to three decades. At present, more than half of the publications are in the field of toxicology and environmental toxicology, and less than

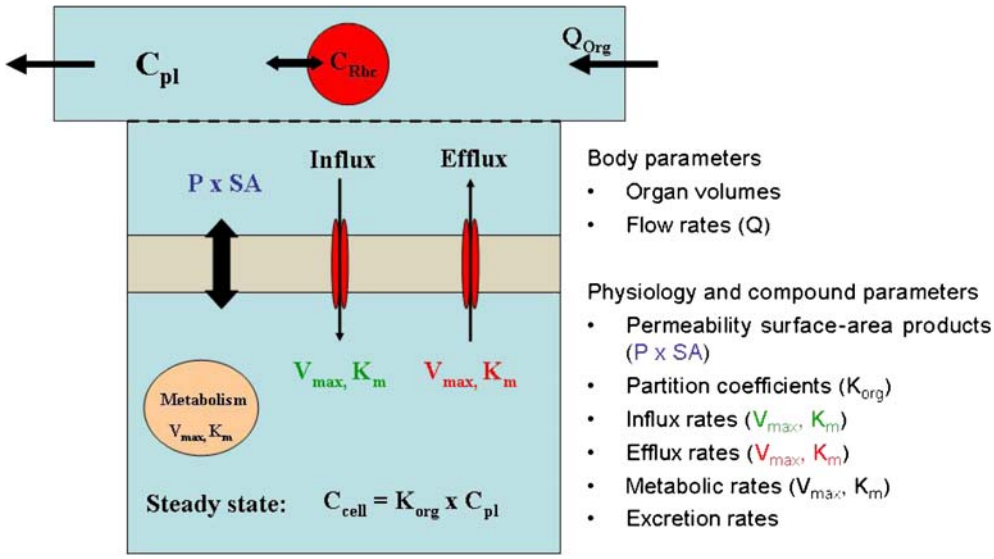

Fig. 2. Important mechanisms for the intra-organ distribution of small molecules. (C_{Rbc}) Concentration in red blood cells, (C_{pl}) Plasma concentration, (Q_{Org}) Organ flow rate.

10% describe drug development (5). The majority of all publications within drug development describe small molecule applications where the PBPK model is used for prediction and less for mechanistically understanding drug pharmacokinetics.

A few examples from the pharmaceutical industry have been presented in whch PBPK modelling has been used to predict pharmacokinetic parameters, such as bioavailability and volume of distribution of small molecules in animals based on drug physiochemical data and plasma concentration-time data in humans from animal data (16). Within clinical drug development, PBPK models have been used to extrapolate from healthy volunteers to patients by including the patho-physiological changes associated with the disease into the PBPK model. This approach is a potential powerful tool for extrapolation between Phase I and Phase II; however, difficulties in describing the disease-related physiological changes are complicating the practical use (5,17,18).

A number of covariate factors, such as height, weight, age, race, and gender, are known to affect drug pharmacokinetics. It is also known that blood flow and body composition, for example, vary with age, race and gender and, therefore, have potential impact on the PBPK of inter-individual variability on pharmacokinetics. Special emphasis was put on PB-PK/PD modelling in sub-populations, such as children, obese individuals, and renally impaired patients. Studies on ciprofloxacin and paclitaxel showed that the predicted variability in virtual population (age-, gender-, weight-, and height-matched) reflects the variability observed *in vivo*, and whole-body PBPK facilitates population predictions instead of post-hoc analysis. It was demonstrated that whole-body PK/PD modelling was a powerful tool for investigating the influence of patho-physiological conditions and drug-specific parameters on pharmacokinetics. When combined with a mechanistic or empirical pharmacodynamic model, the therapeutic outcome or adverse events within a certain population could be obtained prior to clinical studies. The work was part of the scientific basis for the development of the software PKSim for physiologically-based PK/PD modelling (5,15).

Modelling Small Peptides

The pharmacokinetics of the first cyclosporine, Cyclosporine A, a cyclic lipophilic polypeptide of 11 amino acids, mainly used in transplantation therapy, was studied by Kawai using a PBPK model assuming perfusion-rate-limited tissue distribution and assuming that each tissue acted as a well-stirred compartment (7,12). Cyclosporine A can be regarded as an example of bridging the small non-protein molecules (Mw< 1000 Da) and the macromolecules, like growth factors and antibodies, with regard to PBPK modelling. Although Cyclosporine A is a lipophilic peptide, the initial PBPK model developed did not provide a sufficiently accurate description of the pharmacokinetics in blood and tissue even though the unbound equilibrium distribution ratios, as well as the values of the fraction unbound, and the distribution isotherm of cyclosporine between erythrocytes and plasma were included in the rate equations describing the time course of the drug concentration in each tissue (12). Subsequent model development was performed where the transport of the cyclosporine across cell membranes, affinity to plasma proteins and active membrane transporters were included to describe drug transport between various physiologic compartments, i.e. blood (as PK monitoring site), tissue-intracellular space for organs of either efficacy (e.g. graft) or adverse effect (e.g. brain) (8,12).

By studying cyclosporine derivatives, the impact of carrier-mediated transmembrane transport, passive diffusion and intracellular binding on the disposition kinetics was described and demonstrated that a PBPK model containing organ-specific transmembrane transport and intracellular binding were well-suited to provide insight into complex disposition kinetics. The model was developed based on rat experiments and subsequently scaled up to dogs and humans by modifying physiological parameters, tissue distribution and elimination clearances. Although large differences in metabolic clearance were observed, the interspecies scaling correlated well with the subsequent experimental data from dogs and humans (7). The model was used to assess pharmacokinetic and pharmacologic responses of cyclosporine A in various patient populations, as

well as to design new derivatives of cyclosporine A by predicting the differences in PKPD properties from the original drug (12).

The cyclosporine PBPK modelling example showed that for large drugs that penetrate through the cell membrane in a non-instantaneous time-dependent manner, the intracellular target concentration is not simply predictable from the blood pharmacokinetics. The intracellular specific binding and the membrane transporters cause a complex non-linear kinetics that can complicate clinical interpretation of PK/PD data. The PBPK modelling approach can provide a mechanistic insight into these complex systems (8).

Computer-Aided Physiologically-Based Pharmacokinetic Modelling

The use of personal computers for PBPK modelling has been a pivotal element in implementing the PBPK approach in drug research and development within the last few decades. Several software tools for PBPK modelling are available and can be divided into three groups: 1) general modelling and simulation software typically used by professional modellers with an in-depth programming and statistical knowledge of modelling, 2) software that models or simulates specific processes within pharmacokinetics, such as absorption or metabolism and 3) software dedicated to generic whole-body PBPK modelling and simulation. Common to the two latter groups are that these software are typically developed for commercial use and are generally more user-friendly and require less programming and statistical modelling skills. All current in silico PBPK modelling examples are based on small to intermediate sized molecules. None of the commercial software tools have been tested or validated for PBPK modelling of macromolecules (15). One unpublished attempt to validate the absorption of proteins using GastroPlus® was presented by Kristensen. GastroPlus is a software tool developed for predicting the intestinal absorption. It is based on a semi-physiological model—the Advanced Compartmentalized Absorption and Transit (ACAT) model—that divides the entire GI tract into nine different compartments, where rate and extent of absorption is estimated as a function of time based on compound lipophilicity and pH-dependent solubility and permeability (19). The validation results showed that GastroPlus was not able to predict the intestinal absorption or the subsequent kinetics of proteins and peptides due to improper handling of the pH-dependent properties of the molecules. However, since GastroPlus also assumes that the absorption is passive, no account is taken of active transport processes, including both uptake and efflux transporters. Other absorption-specific software tools, like iDEA, have the same limitations towards macromolecules as GastroPlus and must be judged unsuitable for estimating macromolecular absorption.

PHYSIOLOGICALLY-BASED MODELLING AND PREDICTION FOR MACROMOLECULES

General Aspects of PBPK Modelling of Macromolecules

Although the majority of publications with PBPK modelling are based on small molecules (Mw<1000 Da), more publications are now appearing where PBPK modelling and simulation are applied to macromolecules (5,20).

The overall concepts of PBPK modelling are still valid for macromolecules, but a number of factors need to be considered: i) The molecular size and polar nature of macromolecules mean that the permeability across cell membranes is low compared to small molecules. This means that physico-chemical parameters such as lipophilicity and pH-dependent charge will only have insignificant importance for the disposition of macromolecules. Instead, a slower and more limited distribution should be expected involving mainly convection rather than diffusion through pores in the vascular endothelium of the various organs involved in the drug disposition. ii) The lymphatic system is believed to play a more prominent role in absorption and disposition of macromolecules compared to small molecules due to the structure of the lymphatic capillaries, where the size of the lymphatic vessels is much larger than the paracellular pores in the vascular endothelium. This means that macromolecules will predominantly be transported by convective movement through the lymph and enter the vascular circulation via the central lymphatic ducts. iii) Macromolecules will also frequently interact with specific binding proteins involved in their transport and regulation. A wide range of macromolecules such as growth hormone, cytokines and growth factors are associated with specific binding proteins: The binding proteins may either prolong the circulation time or enhance the clearance of the macromolecules. iv) Site-specific and target-oriented receptor-mediated uptake can also affect the disposition of macromolecules. The molecules bind to the specific receptor and are taken up intracellularly by endocytosis.

With the above-mentioned factors in mind, the PBPK structural model for macromolecules needs to be adjusted compared to small molecules (5,20). The slow and limited distribution of macromolecules and especially of monoclonal antibodies needs to be included in the model. Binding of monoclonal antibodies to the neonatal Fc receptor (FcRn) in the endothelium in various organs (especially skin and muscles) and the subsequent distribution into endothelial cells and finally into the interstitium has significant impact on the distribution and should also be integrated in PBPK modelling. Furthermore, target-mediated disposition (binding to cell surface proteins, internalisation and clearance) should be included. Target-mediated binding and clearance is usually a high affinity, low capacity phenomenon resulting in concentration-dependent clearance at therapeutic concentrations. Therefore, distribution and elimination processes are often interrelated for macromolecules and especially for monoclonal antibodies (21). The development in fluorescence-activated cell sorting (FACS), microdialysis and imaging techniques could potentially enable measurements of macromolecules in the interstitium and on the cells, surfaces and provide data, which could lead to more detailed and improved PBPK modelling. The concepts of local kinetics and dynamics of xenobiotics have been discussed by Pelkonen *et al.* (22)

The study design used to generate data needed to build up a PBPK model for macromolecules does not differ in principle from that of small molecules. However, certain additional aspects may need consideration: 1) the impact of endogenous protein on the total protein concentration and the subsequent pharmacokinetic parameter estimation, 2) the immunogenetic potential of the macromolecule and the

impact of antibodies, both neutralising and non-neutralising antibodies, on the pharmacokinetics, 3) the circadian rhythm or diurnal variation in production of endogenous macromolecules and the impact on pharmacokinetics, and 4) therapeutic proteins that may exhibit different pharmacokinetics and pharmacological or toxicological effect depending on the rate and/or route of drug administration. These issues have been discussed in detail by Mahmood and Green (23).

A few examples of PBPK modelling of antibodies exist. The first attempt to generate a PBPK model for an antibody was presented by Covell in 1986 (24). Six organs were included in the model. Uptake into tissue from plasma was assumed to take place via diffusion or convection. This model was later refined by Baxter *et al.* (25) to include a tumour compartment in the model and introduced the concept of "two pore formalism" to the model. In this model, immunoglobulin G (IgG) diffusion was negligible through large and small pores in the body. In 2005, Ferl *et al.* (26) presented the first attempt to model FcRn binding. However, since FcRn was only modelled in skin and muscle tissue, it could not account for FcRn activity in other tissues. Garg and Balthasar presented a PBPK model (13) where uptake in tissue via convection and via endocytosis into vascular endothelial cells were included. IgG was fitted to bind to FcRn in all tissues and mediated protection from catabolism, recycling to plasma and transcytosis to the interstitial fluid. The model provided a more accurate prediction of antibody disposition in both normal wild-type and FcRn-knockout mice, although the concept of "two pore formalism" was not included. In order to investigate some of the disagreement between experimentally observed and PBPK model-predicted data with respect to the role of FcRn in antibody disposition, Balthasar and coworkers inhibited the FcRn with IVIG treatment in control and FcRn knockout mice (27). The rationale was that potential differences between observed and predicted data were due to some of the simplifying assumptions with regard to convective transport and lymphatic flow. By inhibiting FcRn in a dose-dependent manner, a better understanding of the role of FcRn in tissue distribution of IgG could be achieved. The results showed that clearance of IgG increased with increasing dose of IVIG. The subsequent modelling of data indicated that fluid phase endocytosis and FcRn-mediated transport account for a significant fraction of the distribution of IgG to peripheral tissue.

Interspecies Scaling of Pharmacokinetic Parameters of Macromolecules

Interspecies scaling of pharmacokinetic parameters can be defined as prediction of the pharmacokinetics of a compound in a species based on information obtained from other species. Interspecies scaling can be performed either by allometric scaling of pharmacokinetic parameters or by PBPK modelling. Allometric scaling attempts to relate a parameter of interest, e.g. volume of distribution, clearance or half-life, to body weight or size by use of a general allometric equation: $Parameter = a \cdot BW^b$, where a is the allometric constant, BW body weight and b the allometric scaling exponent. Whereas a tends to vary dependent on the parameter and compound, b is more restricted to the parameter, and, as a general rule for the volume of distribution, b is assumed to be 1, for clearance b is assumed to be 0.75, and for half-life b is assumed to be 0.25 (28,29).

A number of macromolecules have successfully been scaled by allometric scaling, although cross-species scaling is complicated by species differences in protein-receptor interactions, e.g. binding to FcRn, Fcγ-receptors or target molecules. The fixed allometric exponent value of 0.75 for clearance has also been criticised for being highly erratic and unreliable (30). Grene-Lerouge *et al.* successfully scaled clearance and volume of distribution of digoxin-specific Fab molecules between mice, rats and rabbits (31), and Woo and Jusko scaled erythropoietin (32). For monoclonal antibodies, the situation is more complex, since the drug pharmacokinetics depend on target-mediated disposition, Fcγ-receptors and FcRn, which is shown to be species-dependent. However, for antibodies binding to soluble ligands, e.g. cytokines, the target-mediated disposition and the binding to Fcγ-receptors are often not relevant, leaving the FcRn as the only determinant for antibody clearance. In this situation, Vugmeyster *et al.* (33) showed a successful scaling of clearance and volume of distribution. For monoclonal antibodies binding to cell surface ligands, target-mediated disposition is very common, and binding to Fcγ-receptors may be relevant, whereas FcRn binding is only a minor determinant of antibody clearance. In this situation, allometric scaling is highly uncertain and can not be used to estimate clearance and volume of distribution.

PBPK modelling has been used as an alternative to allometric interspecies scaling for several small molecules. A direct comparison of 19 compounds showed that the pharmacokinetic parameters and plasma concentration-time profiles were better predicted in humans from animal data using the PBPK model approach than the traditional allometric approach (16). The PBPK model developed for cyclosporine was also used for interspecies scaling between rats, dogs and humans, as previously mentioned (7).

For macromolecules, especially interspecies, scaling of monoclonal antibodies using a PBPK model has been described. Baxter *et al.* were among the first to present examples of interspecies scaling using a PBPK model where non-specific antibody clearance, FcRn expressing organs, target expressing organs and lymphatic transport were incorporated (25). Others have followed, and today we also have examples of intra-species scaling, where PBPK models have been used to scale antibody kinetics between antigen expressing and non-antigen expressing mice, and wild type *versus* FcRn-knockout mice (13).

Integrating PD in the PBPK Models of Macromolecules

Target-mediated disposition models are used to describe the interaction between macromolecules and target antigens with the main focus on the kinetics of the target expression and the implication for the PK/PD relationship of the macromolecule.

An example of mechanistic modelling of target binding of two therapeutic antibodies was presented by Lowe. Xolair, a monoclonal antibody against immunoglobulin E (IgE) used for the treatment of atopic disease, was modelled with respect to binding of antibody and IgE, enabling prediction of free and total IgE in plasma. The IgE turnover parameter estimates obtained by the model were compared to literature

data of direct measurement. The model was developed based on clinical data from Japanese patients and used to predict free and total IgE concentrations in Caucasians.

In a second example, Lowe investigated an antibody against interleukin-1β (IL-1β), ACZ885 used for treatment of inflammatory disorders. In the model, a two-compartment distribution model was integrated with target binding. IL-1β was subsequently linked with clinical measurements of disease state. The model allowed predictions of free and total IL-1β concentrations as well as that of C-reactive protein and the drug concentration itself. An increased physiologic and biochemical realism in the models enables the ability to predict not only pharmacokinetics, but also the pharmacodynamics of selected system components even though they may not be directly measurable.

A whole family of PK/PD binding models should be developed during the drug development phases. In the early discovery phase, estimating dose and defining binding affinities are required given target localisation, expression levels and turnover. In the preclinical phase, the *in vitro* and *in vivo* binding affinities, comparability is assessed and the relevant non-human species (if any) is identified. In the clinical phase I, target binding is verified in humans and it is confirmed whether suppression of free target and/or formation of complexes is feasible. In phase II, the binding models are assessed in patients. Expression levels and competing factors may not be the same as in healthy individuals. Phase II should enable building a relationships between target binding and clinical endpoints. Phase III should confirm the binding model. A population model should be used to collate multi-study information. The circle should be closed by returning information to Discovery for development of future binding models.

Overgaard presented a case-example of how integrating a binding model with an antibody PK model was used for PK/PD modelling and simulation in order to provide a rational and cautious selection of a first human dose of a therapeutic antibody. The strategy was to combine *in vitro* binding data on human cells; PK/PD data in transgenic mice, where target binding was the pharmacodynamic part; PK data from monkeys; and literature human antibody PK data to form a predictive PK/PD model in humans.

A significant target-mediated drug disposition was observed in transgenic mice leading to a two-compartment model with a non-linear dose-dependent elimination. However, since typical human antibody PK data from the literature gave the most conservative exposure estimates, it was adopted for the human simulations. The target binding model developed in transgenic mice was used for modelling the human binding since it was consistent with the *in vitro* human data. In this example, information generated during the discovery and preclinical phases of drug development was used to build a model for a rational dose selection of a first human dose.

Extensive work on the PK/PD relationship of erythropoietin (EPO) had been performed by Jusko and co-workers (34, 35). A PK/PD model for predicting the disposition and dynamics of EPO was presented as an example of the importance of a good mechanistic PD model in understanding a complex cascade of pharmacodynamic events. The absorption of EPO after subcutaneous administration was shown to be slow, incomplete and dose-dependent. The disposition was nonlinear over a wide range of doses. A dual-absorption rate model was included to account for the absorption. The disposition was described by a partial receptor-mediated disposition model that gave a more comprehensive description of the pharmacokinetics in humans. Since the pharmacodynamics of EPO can be described by monitoring reticulocytes, red blood cells and haemoglobin in blood, this was initially modelled by a stimulatory indirect response model. However, this approach failed to describe the PK/PD of EPO. Instead, the natural cell lifespan concept was used and combined with the use of transduction compartments to reflect both precursor pools and variability in number and lifespan of serial progenitor pools. To account for tolerance behaviours observed after multiple dosing with EPO, a feedback mechanism affecting early progenitor pools was added to the initial model. The result was a second generation PK/PD model that recognised several determinants of EPO and allowed quantification of data from rat, monkey and human after single and multiple dosing.

CONCLUSIONS

A number of publications have appeared within the last two decades documenting the use of PBPK in understanding the mechanism of action and scale-up of macromolecules. Special attention has been given to the PBPK modelling of monoclonal antibodies because of their highly complex pharmacokinetics with non-linear disposition and elimination. The PBPK models have been valuable in understanding the mechanisms behind the complex pharmacokinetics. Major advances have included understanding the significance of convective transport instead of simple diffusion, inclusion of the "two pore formalism," inclusion of specific binding and target-mediated IgG elimination and inclusion of the role of FcRn in IgG transport and elimination.

Other macromolecules such as EPO have been the subject of advanced PK/PD modelling using the natural cell lifespan concept in combination with the use of transduction compartments to reflect both precursor pools and variability in number and lifespan of serial progenitor pools. The models allowed a better understanding of tolerance behaviours observed after multiple dosing with EPO and allowed quantification of data from rat, monkey and human after single and multiple dosing.

During the discussion at the workshop, it was generally agreed that physiologically-based PK/PD modelling of therapeutic proteins and peptides was feasible and desirable. Several issues were discussed: i) The role of the lymphatic system should be considered when absorption after extravascular administration is modelled. ii) Target-mediated drug disposition was regarded as an important feature that should be considered when the PK model is generated. iii) Although the pharmacokinetics are generally well-understood for most drugs, the complexity raises when the pharmacodynamics are added to the model. iv) Complex multi-organ PBPK models may not always be necessary when only a limited number of organs are affected. v) More mechanistic PK/PD models will be relevant when adverse events/toxicity are included in the PK/PD modelling. vi) Physiologically-based PK/PD modelling may be useful in predicting the sample size of special patient subpopulations for clinical studies.

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