

Retinal physiologically based pharmacokinetic modeling of intravitreally administered large molecules across species

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Abstract

This dissertation focuses on improving our understanding of large molecule ocular pharmacokinetics (PK) in different species and bridging the gap between animal models and human studies. The study utilizes physiologically based pharmacokinetic (PBPK) modeling and mechanistic PK modeling to explore whole-body, as well as ocular PK across the three species rabbit, non-human primate, and human.

Initially, this work investigates the translatability of parameters related to neonatal fragment crystallizable receptor (FcRn) binding and target-mediated drug disposition (TMDD) for the therapeutic protein efalizumab. PBPK models were developed for rabbit, non-human primate (NHP), and human, and the parameters were compared between species. The results indicate that while FcRn affinity parameters cannot be translated, parameters related to TMDD can be translated from NHP to human.

Furthermore, the dissertation focuses on the simulation of retinal drug concentrations after intravitreal administration in rabbits, aiming to improve our understanding of ocular physiology and drug distribution in the eye. A mechanistic ocular model was developed for bevacizumab. The model successfully describes the concentration-time profiles in the injected eye, plasma, and fellow eye. It also provides insights into the interpretation of retinal volume and permeability, enabling more accurate predictions of drug exposure in the fellow eye.

Finally, this work explores ocular PK across rabbits, monkeys, and humans, emphasizing the importance of cross-species translation in ocular pharmacokinetic studies. Extended mechanistic ocular models were developed for each species using ranibizumab in a case study. The models successfully describe the PK data in all three species and highlighted inter-species differences in vitreous-to-aqueous diffusion rates and systemic drug disposition.

Overall, this dissertation contributes to the improvement of ocular PK modeling across different species and provides valuable insights into the translation of large molecule models. The findings enhance our understanding of general cross-species PK, as well

as ocular drug PK and support the development of more accurate predictions for human dose estimation and ocular drug exposure.

Zusammenfassung

Diese Dissertation befasst sich damit, unser Verständnis der okulären Pharmakokinetik (PK) großer Moleküle in verschiedenen Spezies zu verbessern und die Lücke zwischen Tiermodellen und Humanstudien zu schließen. Die Studie nutzt physiologisch basierte pharmakokinetische (PBPK) Modelle und mechanistische PK-Modelle, um die Ganzkörper-PK sowie die okuläre PK in den drei Tierarten Kaninchen, Affe und Mensch zu untersuchen.

Zuächst untersucht diese Arbeit die Übertragbarkeit von Parametern, die sich auf die Bindung des neonatalen kristallisierbaren Rezeptorfragments (FcRn) und die Zielprotein-vermittelte Medikamentendisposition (TMDD) des therapeutischen Proteins Efalizumab beziehen. PBPK-Modelle wurden für Kaninchen, nicht-menschliche Primaten (NHP) und den Menschen entwickelt, und die Parameter wurden zwischen den Spezies verglichen. Die Ergebnisse zeigen, dass die Parameter für die FcRn-Affinität nicht übertragen werden können, während die Parameter für die TMDD vom NHP auf den Menschen übertragen werden können.

Weiterhin befasst sich die Dissertation mit der Simulation retinaler Arzneimittelkonzentrationen nach intravitrealer Verabreichung im Kaninchen, um unser Verständnis der okulären Physiologie und der Arzneimittelverteilung im Auge zu verbessern. Es wurde ein mechanistisches okuläres Modell für Bevacizumab entwickelt. Das Modell beschreibt die Konzentrations-Zeit-Profile im injizierten Auge, im Plasma und im nicht-injizierten Auge. Es liefert auch Einblicke in die Interpretation des Netzhautvolumens und der retinalen Permeabilität, welches genauere Vorhersagen der Arzneimittlexposition im nicht-injizierten Auge ermöglicht.

Zuletzt untersucht diese Arbeit die okuläre PK bei Kaninchen, Affen und Menschen, wobei die Bedeutung der artenübergreifenden Übertragung von Ergebnissen aus okulären pharmakokinetischen Studien betont wird. Für jede Spezies wurden erweiterte mechanistische Augenmodelle entwickelt, wobei Ranibizumab als Fallstudie diente. Die Modelle beschreiben die PK-Daten aller drei Spezies und zeigen die Unterschiede zwischen den Spezies in Bezug auf die Diffusionsraten vom Glaskörper in das Kammerwasser und die systemische Medikamentendisposition auf.

Insgesamt trägt diese Dissertation zur Verbesserung der okulären PK-Modellierung in verschiedenen Spezies bei und liefert wertvolle Einblicke in die Entwicklung von Modellen für große Moleküle. Die Ergebnisse verbessern unser Verständnis der allgemeinen speziesübergreifenden PK sowie der okulären Arzneimittel-PK und unterstützen die Entwicklung genauerer Vorhersagen für die Dosisabschätzung beim Menschen und die okuläre Arzneimittelexposition.

Chapter 1: Background

Motivation

Medical need of ocular therapy

Worldwide more than 1 billion people suffer from visual impairment and among them more than 40 million are blind. The prevalence in low- and middle-income countries is higher, however, population ageing also increases the risk of ocular disorders and diseases in high-income countries (Burton et al., 2021). A big subgroup of ocular diseases are diseases of the retina, the part of the eye that contains the neurons for vision. Among blurry vision and vision loss these diseases may cause other symptoms like eye pain, redness, and sensitivity to light. There are rare inherited retinal degenerations that occur at a young age. However, most patients suffer from these diseases due to age, and in our ageing population, more and more people will be affected (Wong et al., 2014). Common retinal diseases include diabetic retinopathy, retinal vein occlusion and age-related macular degeneration (Amo et al., 2017). One type of AMD, so-called choroidal neovascular age-related macular degeneration or "wet AMD," is the main cause of blindness in older people (Bressler, 2004). The disease is highly influenced by vascular endothelial growth factors (VEGF). These cause the formation of highly permeable blood vessels in the retina, leading to damage of the neural cells which are important for vision (Rosenfeld et al., 2006; Penn et al., 2008).

The severe effects and increasing prevalence of retinal diseases makes it necessary to fully understand ocular physiology and to develop medication that can prevent and alleviate ocular complications. Against the backdrop of an ageing population, the market for ophthalmic drugs is growing rapidly and recent analyses show an increase in drug approvals of 2.2 per 5-year quartile, underlining the importance of ocular drug development (Gu and Janetos, 2021). The research on these medications can be supported by improving the understanding of physiological aspects and drug properties relevant for ocular disposition.

Ocular anatomy and physiology

The eye enables vision by capturing light signals and converting them into interpretable information for the brain. Several parts of the eye are involved in this process: cornea, aqueous chamber, iris, lens and ciliary body, vitreous body, retina, choroid, sclera and optic nerve (see Figure 1).

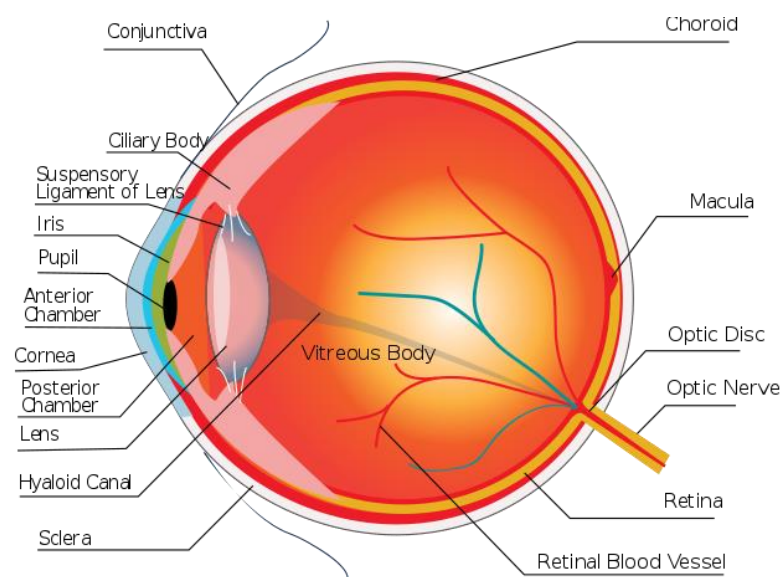


Figure 1. Human eye anatomy. Figure available at Wikimedia Commons (downloaded 2024-06-04, https://commons.wikimedia.org/wiki/File:Eyeball_dissection_hariadhi.svg, author: Hariadhi).

When light enters the eye, it first hits the cornea, a transparent body that refracts the light and allows it to converge on its way through the adjacent aqueous chamber. The aqueous chamber is filled with aqueous humor, a liquid with a composition similar to blood plasma. It is produced and secreted by the ciliary body, and then drained via the Schlemm's canal into systemic circulation (Loftsson et al., 2008).

From the aqueous chamber, the incoming light is directed through the pupil, an opening surrounded by the iris. With the help of its smooth muscles, the iris can adjust the size of the pupil depending on the intensity and availability of the light. Once the light has passed through the pupil, it reaches the lens, a structure made up of proteins

known as crystallins. It can change its shape to focus on objects at different distances, improving the image of the cornea (Litzinger and Rio-Tsonis, 2013). The light signal further passes the vitreous humor, a clear gel composed of collagen and hyaluronic acid that fills up the vitreous body of the eye (Loftsson et al., 2008). It contains the hyaloid canal, a glial sheath that starts in the dorsal region of the vitreous body, which is adjacent to the retina, and extends ventrally towards the posterior pole of the lens. The entire posterior wall of the vitreous chamber is covered by the retina, which captures the light. It contains photoreceptors which transmit the incoming stimulus to the optic nerve, through which it finally reaches the brain for interpretation (Litzinger and Rio-Tsonis, 2013).

In addition to photoreceptors, the retina contains different neural cells and ganglion cells. It is supplied with nutrients by the retinal blood vessels. Two structures delimit the retina: The inner limiting membrane (ILM) forms the structural boundary towards the vitreous. It mainly consists of a network of collagen type IV, laminin and fibronectin (Halfter et al., 2008). On the posterior side, the retina is covered with the retinal pigment epithelium (RPE), a cellular monolayer with tight intercellular junctions (Peynshaert et al., 2018). Behind the RPE lie the Bruch's membrane, an elastin- and collagen-rich penta-laminar structure (Booij et al., 2010), and the blood vessels of the choroid. The entire posterior part of the eye is surrounded by the choroid and the sclera (Loftsson et al., 2008).

These structures play a vital role in facilitating the supply and proper functioning of the retina. Considering the crucial function of the retina in vision, the importance of retinal diseases and their treatment is obvious. The development of medication for such treatments, however, is difficult due to the low accessibility of the retina, which is located far from the administration site, and is further complicated by our limited understanding of ocular physiology and its effect on drug disposition. In contrast to diseases of other organs, which are often easily accessible via the bloodstream, the retina can only be reached via adjacent tissues. Thus, ocular drug development relies on an understanding of ocular drug PK.

Ocular therapies

The treatments for retinal diseases include small molecule drugs, as well as large molecule drugs, such as therapeutic antibodies and antibody fragments. While small molecules more easily diffuse through tissue barriers, enter cells, and have a shorter ocular half life, tissue accessibility for large molecules is limited due to their size, causing diffusion and elimination processes to occur more slowly (Amo et al., 2017; Lobo et al., 2004). In recent years, therapeutic antibodies have become the leading category of newly developed drugs and have become the top-selling drugs on the pharmaceutical market (Lu et al., 2020). Even though they have only been in use for about 40 years, the development of therapeutic antibodies is very promising and continues to evolve rapidly. In 2018, eight biologics were among the top ten of the best-selling drugs worldwide and the global market for antibody therapeutics was estimated at 115.2 billion USD. Until 2025, it is expected to reach 300 billion USD. New protein therapeutics have been approved for various disease conditions, highlighting their advantages compared to small molecule drugs. Due to their high target specificity, they have fewer adverse effects than small molecule drugs (Lu et al., 2020). Furthermore, they have a longer duration of action, allowing for less frequent administration (Amo et al., 2017). In ocular therapy, therapeutic antibodies and their fragments are commonly administered by an intravitreal (ivt) injection into the vitreous humor of the eye. As this can be unpleasant for the patient, a reduction of administration frequency is favorable. From the injection site, the drugs are supposed to access the back of the eye to achieve an effect in retinal segments (Ranta et al., 2010). Ranibizumab (Lucentis®, Genentech Inc., San Francisco, CA) is a recombinant humanized monoclonal antibody fragment (Fab), directed against VEGF. It has been shown to be an effective treatment for wet AMD, halting the progression of the disease or even curing it (Ferrara et al., 2006). Another anti-VEGF agent is Bevacizumab (Avastin®, Roche, Basel, Switzerland), an immunoglobulin (IgG) monoclonal antibody (mAb) originally developed for oncology therapy and used off-label in ocular therapy (Grisanti and Ziemssen, 2007).

Current challenges in ocular drug development

Due to the anatomical and physiological complexities of the eye, ocular drug development faces various challenges. The fact that the retina is not easily accessible for sampling and its location far from the injection site, makes it difficult to predict retinal exposure. Our understanding of the pharmacokinetic mechanisms in the eye is still limited, especially in humans, and drug availability at the target site is strongly influenced by physiological barriers (Amo et al., 2017). The permeabilities of these barriers are not described well in literature and thus, need to be estimated from PK studies. Since retina samples cannot be obtained from humans, PK studies rely on samples from animals to estimate relevant permeabilities. Furthermore, not only is retinal sampling problematic, but even obtaining samples of aqueous and vitreous humor from human subjects is rare and inconvenient. Thus, due to the overall limited availability of human data, there is a general reliance on animal data.

Moreover, inaccurate dosing in ocular experiments is further complicating PK study analysis. An unintentional reduction in the intended dose might occur if the drug oozes out of the eye because of intraocular pressure, an observation also documented in other publications (Amo and Urtti, 2015; Bussing and Shah, 2020).

A further challenge is the lack of knowledge regarding the blood-ocular barrier. After ivt administration, a drug diffuses from the vitreous to the surrounding ocular tissues and is eliminated from the eye into the systemic circulation. This elimination occurs via the outflow of aqueous humor and via the blood-ocular barrier (Amo et al, 2017). It is crucial to determine the amount of a drug that enters the bloodstream as, firstly, systemic exposure can lead to side effects and, secondly, blood data is often the only available source to draw conclusions for human ocular drug PK. Moreover, to accurately describe systemic PK, it is not enough to understand the eye-blood barrier. Systemic distribution and elimination processes need to be determined. This can be done by analyzing drug PK after iv administration or by performing in-vitro experiments determining relevant ADME processes. However, in the case of ocular drugs, there is often a lack of iv data or in-vitro data (describing e.g., target-binding kinetics) for both humans and animals. This further adds to the challenges faced in developing effective ocular medications.

Understanding ocular drug delivery and pharmacokinetics is crucial for both, existing retinal therapeutics, and the development of new medications. Additionally, inter-species differences of drug pharmacokinetics play an important role, as human eye segments cannot be directly studied. Rabbits and monkeys are both relevant pre-clinical species for ocular studies (Grossniklaus et al., 2010; Mage et al., 2019). Monkeys additionally exhibit a high sequence homology to humans regarding endogenous proteins and overall similar anatomical body structures (Germovsek et al., 2021).

Computational modeling, and in particular PK modeling, is an effective tool for understanding the drug PK within the eye (Himmelstein and Lutz, 1979; Danhof et al., 2008; Missel and Sarangapani, 2019). Given that there is a mechanistic understanding of the physiology of the studied system, drug concentration-time profiles can be simulated in tissues which are not accessible for PK sampling. Moreover, physiological differences between animal species can be considered. Putting the eye within a systemic context by taking into account not only ocular PK, but also systemic PK of a drug, offers the opportunity to improve the understanding of the blood-ocular barrier and predictions from sparse data (e.g., only plasma data). The following subchapter illustrates the value of computational modeling for ocular PK.

Computational Modeling

The utilization of computational modeling is increasingly prominent in life science disciplines. It led to a revolution in our understanding of complex biological systems and their relationship to human health. Biological processes that are difficult to study directly can be analyzed through computational techniques, supporting the understanding of diseases and their personalized therapy (Ji et al., 2017).

Computational modeling is usually achieved by several steps which consist of model development, validation and refinement. An initial model structure is developed, and subsequently validated by comparing the modeling outcome to experimental data. Then the model is refined to best describe the available data upon calibration of the model parameters. To support parameter estimation in dynamic, ordinary differential

equation (ODE)-based models, optimization algorithms can be utilized to find the best parameter values. These algorithms try to find parameter values minimizing the differences between model predictions and observed data. Another way of improving the model fit is adjusting the model structure. Discrepancies in model predictions and observed data might be attributable not only to model parameter values, but also to an incomplete understanding of the studied system. By adding or refining processes in the model structure, the model can be adjusted to better describe observed data (Collin et al., 2022; Ji et al., 2017). A model that is able to describe the observed data which it was calibrated to (the so-called training data), and ideally also data that it was not exposed to yet (so-called validation data or test data), can be used to make predictions for various scenarios and support research for the system under investigation, e.g., hypothesis testing, experimental design, and decision making (Collin et al., 2022). Despite the difficult task of determining mechanistic systems equations that enable precision medicine, the advances in computing power and the increasing availability of precise biological data, in iterative interaction with powerful mathematical models, allow for the data-driven identification of equations that can describe the complexities of living systems (Gomez-Cabrero and Tegnér, 2017).

Compartmental and non-compartmental pharmacokinetic modeling

When developing new medications, it is important to understand drug absorption, distribution, metabolism and elimination (ADME) in order to dose the compound correctly and achieve the desired effect. If the in-vivo PK of a drug is not properly understood, its successful clinical application will be difficult. PK modeling is used to improve the understanding of the in-vivo ADME of a drug and is therefore a valuable tool in drug development (Zou et al., 2020).

In PK modeling, the ADME of a compound in a certain physiological system are expressed through mathematical equations. These equations can vary in complexity, depending on the specific system under investigation. They may rely on assumptions or biological relationships that have been established through experimentation or

adherence to physical principles (Derendorf and Meibohm, 1999; Sheiner and Steimer, 2000) .

A common representation of a biological system is a system of differential equations (ODEs). ODEs can describe the time course of a biological quantity, such as the drug concentration, at a certain location. They can also capture more complex, non-linear relations of a biological system by depicting the interactions of its components (Hunt et al., 2013).

The two main classes of pharmacokinetic models are non-compartmental and compartmental models. While non-compartmental models do not assume spatial differences within the system, compartmental models divide the body into physical spaces that represent a structure with certain drug distribution characteristics, which are not necessarily referring to defined functional entities such as tissues or organs. One-compartment models assume that the drug disperses uniformly throughout the body, while two-compartment models separate the body for instance into a central and a peripheral compartment to account for more complex distribution kinetics. The kinetic processes of a model, e.g., the drug transfer between compartments, its metabolism and elimination, are described by rate constants. The complexity of a model can vary, ranging from a simple structure to a very detailed and mechanistic representation achieved by incorporating additional relevant processes and compartments (Holz and Fahr, 2001).

By providing a comprehensive understanding of the processes impacting the drug's concentration-time profile in the body, PK modeling can optimize therapeutic regimens and determine the efficacy of a medication, as well as its toxicity. It can define appropriate dosing intervals in pre-clinical and clinical trials to guarantee treatment success while avoiding adverse effects (Zou et al., 2020).

PBPK modeling

One example of PK modeling with a very detailed and mechanistic structure is physiologically based pharmacokinetic (PBPK) modeling (Kuepfer et al., 2016). While classic mechanistic models do not necessarily relate their compartments to functional

entities, the compartments of a PBPK model correspond to well-defined structures or organs, which are interconnected by physiologically interpretable fluxes such as blood flow or lymph flow (Holz and Fahr, 2001). A drug can be applied into this system of interconnected compartments by different ways of administrations, as depicted in Figure 2.

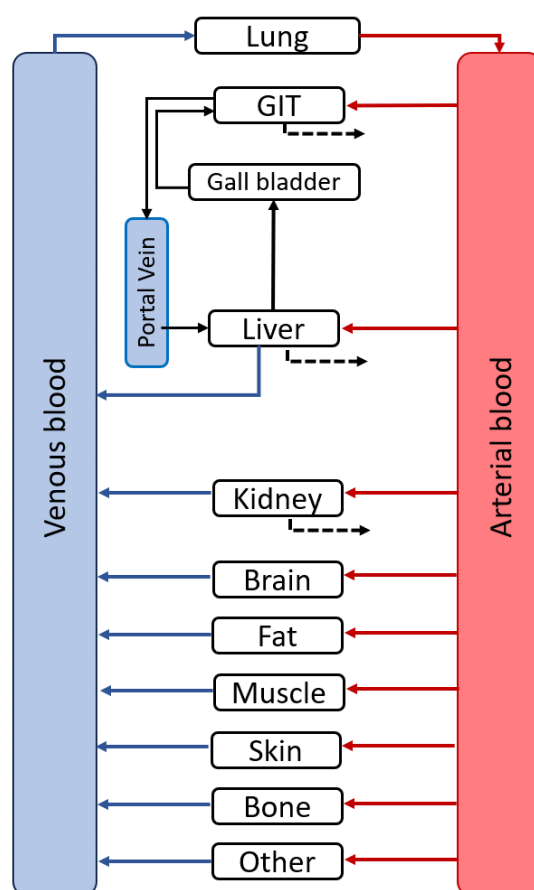


Figure 2. Graphic representation of a human whole-body PBPK model. Organs are represented by compartments and connected by venous and arterial blood. Solid lines represent blood flow pathways, dashed lines represent elimination pathways. GIT: gastrointestinal tract.

These interconnected compartments are further characterized by their physiological properties, such as a specific volume, a certain abundance of endogenous proteins (e.g., a drug target), or a defined barrier to the adjacent compartment, that regulates

the movement of substances between these compartments. In this way, PBPK modeling enables a more thorough explanation of absorption and disposition processes, considering tissue characteristics and the interplay between various drug PK processes. It uses information on the physico-chemical properties of a drug, along with the anatomy and physiology of the species under consideration. Each model parameter of a PBPK model refers to a certain physiological process. Specific parameters can be estimated from data, or, due to the possibility of their physiological interpretation, derived from experiments (e.g., in-vitro measurements of binding constants for substance interactions). While drug-specific parameters require prior knowledge or assumptions, generic software tools provide information on the anatomical and physiological parameters of certain species (Willmann et al., 2007; Thiel et al., 2015; Kuepfer et al., 2016; Niederalt et al., 2018; Mavroudis et al., 2018).

The main applications of PBPK modeling include the optimization of dosing regimens, decision making for formulation strategies and the prediction of drug-drug interactions. Furthermore, PBPK modeling is used to assess drug exposure in certain tissues, as well as drug toxicity and drug safety under certain conditions. Due to the detailed description of an individuals' physiology, it is possible to make personalized predictions, as well as extrapolations to special populations such as infants, elderly, pregnant women, or patients. For these analyses, specific parameters (e.g., organ volumes, changes in physiological function due to disease/age/pregnancy, differences in protein expression) are adjusted in the model, or processes that are only relevant in certain populations (e.g., placental exchange in pregnancy, tumor growth in cancer patients) can be added to the model structure. Thus, drug exposure can be described for individual cases.

Moreover, PBPK modeling can be applied for different species by using species-specific information on anatomy and physiology. Thus, it allows for the application of cross-species translation, where simple approaches like allometric scaling often do not provide adequate predictions (Ward et al., 1999; Mahmood, 2009; Jairam et al., 2018). By making use of physico-chemical and physiological information, and integrating experimental findings, PBPK modeling offers a strong tool to describe pharmacokinetic data and provide insights into the fundamental processes that govern drug PK.

Systemic pharmacokinetics of large molecules

The physiological processes that determine the ADME of large molecules are very different from those for small molecules. The size of large molecules hinders their ability to cross certain barriers or undergo metabolism like small molecules. Consequently, it is crucial to take the PK processes into account that are specific to large molecules when developing respective models.

Large molecule therapeutic drugs have gained increasing importance in the treatment of various diseases. They find application as monoclonal antibodies, as well as antibody fragments engineered for customized pharmacokinetic properties, in various fields, such as oncology, immunology, and retinal health (Holliger and Hudson, 2005; Wang et al., 2008; Amo et al., 2017). The disposition of therapeutic proteins is confined to plasma, interstitial space, endothelial cells containing endosomes, and lymph vessels as depicted in Figure 3.

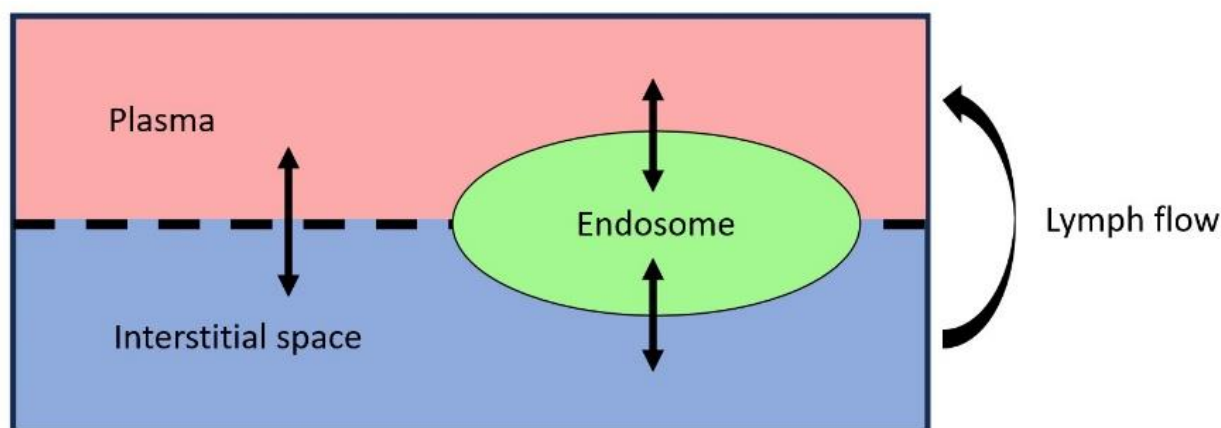


Figure 3. Simplified representation of large molecule disposition into plasma, interstitial space, endothelial endosomes and lymph.

Large molecule PK is characterized by the following processes, many of which have little to no impact on the kinetics of small molecule drugs (Lobo et al., 2004): due to their size, they are generally unable to overcome tight barriers, e.g., cell membranes, so that they are mainly distributed in the plasma and in the interstitial space of a tissue, whereby an exchange between these two sub-compartments can take place across the vascular endothelium through convection and diffusion. However, there is an uptake way into vascular endothelial cells by pinocytosis. Within endothelial cells the endosomal degradation of large molecules takes place. Molecules containing an Fc part, namely IgGs and Fc fusion proteins, can bind to the neonatal Fc receptor (FcRn) protecting them from degradation, and can then be recycled back into plasma or interstitial space. Apart from endosomal degradation, large molecules may be cleared renally, if their size does not exceed a threshold of approx. 69 kDa (Li et al., 2017; Zhao et al., 2012). In addition to the exchange processes between plasma and interstitial space, large molecules can enter the bloodstream from the tissue via the lymph flow. Furthermore, the ability of large molecule drugs to bind to a certain target (see Figure 4) leads to target-mediated drug disposition (TMDD).

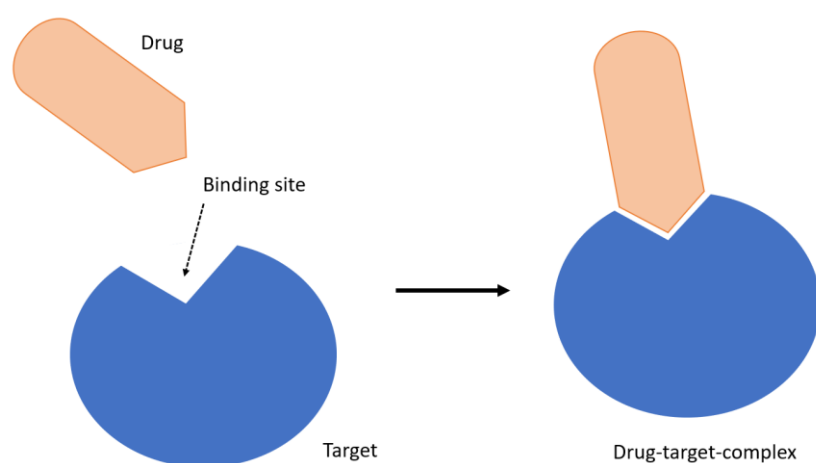


Figure 4. Large molecule drugs bind specifically to the binding site of a target, forming a drug-target-complex.

As a drug undergoes TMDD, its disposition is affected by target-binding and elimination of both, the free target and the drug-target-complex, resulting in a distinct shape of the concentration-time profile that depends on the administered dose, as depicted in Figure 5.

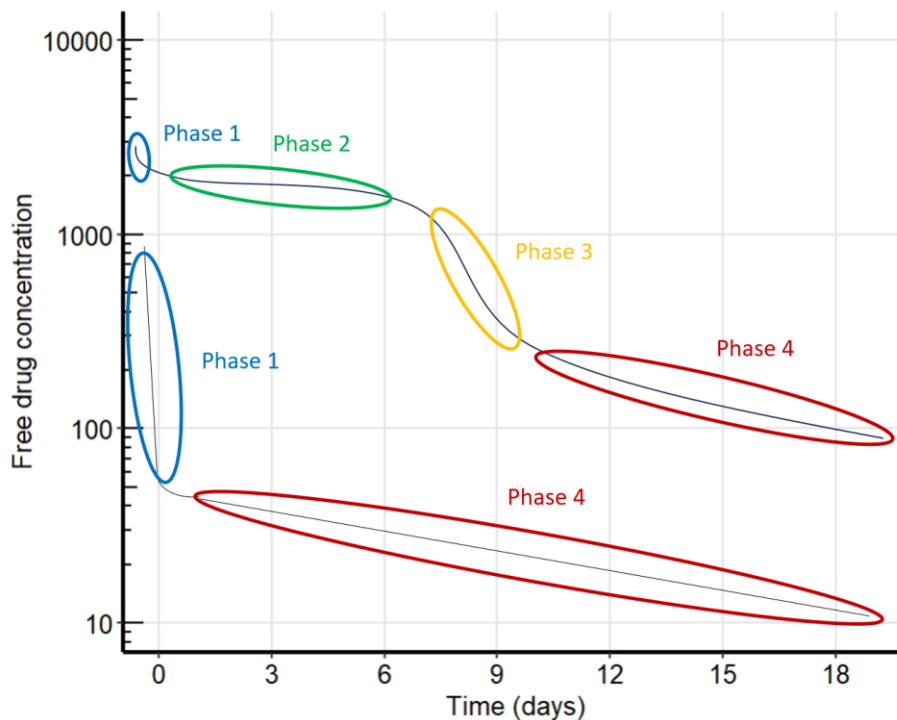


Figure 5. Target mediated drug disposition. Phase 1: Rapid initial drop where drug binds to the free target. Phase 2: Linear phase dominated by the endosomal degradation of the free drug. Phase 3: Transition phase where the target is no longer saturated. Phase 4: Terminal linear phase where the drug is mainly eliminated through the target.

The PK processes can be divided into four phases (Peletier and Gabrielsson, 2012):

1. An initial rapid decline, during which the drug binds to the available target molecules
2. A linear phase characterized by the degradation of the free drug in the endosomes

3. A transition phase, where the target is no longer saturated
4. A terminal linear phase, where the drug primarily undergoes elimination through the target

OSP suite large molecule model

The OSP suite large molecule PBPK model structure is implemented in PK-Sim (Niederalt et al., 2018). A schematical representation is depicted in Figure 6.

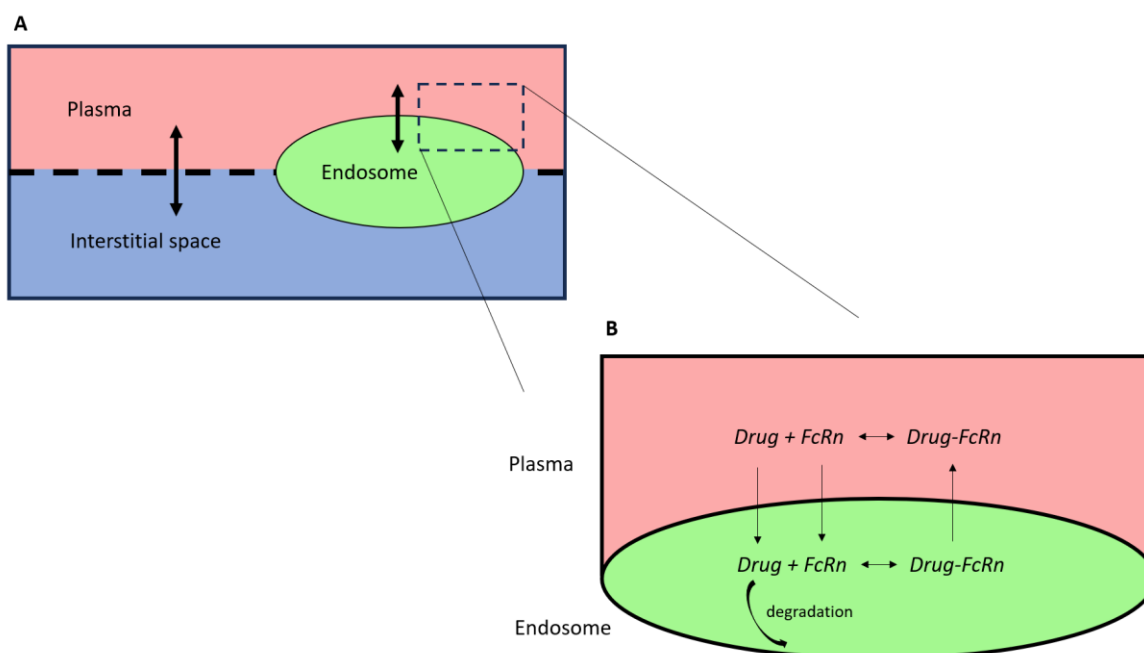


Figure 6. PBPK model structure for large molecules as implemented in PK-Sim (Niederalt et al., 2018). (A) Inter-compartment exchange is assumed between plasma and interstitial space, and between plasma and endosomes. (B) FcRn-binding takes place in plasma and endosomes.

It encompasses various physiological and biochemical processes that govern the disposition of large molecules, including:

- **Transcapillary exchange:** The drug moves between plasma and interstitial space through small and large vascular pores via convection and diffusion.

This process is represented by the two-pore formalism which describes the movement of solutes through pores with different sizes considering the size of the solute, as well as the properties of the pores (Rippe and Haraldsson, 1987; Rippe and Haraldsson, 1994). Factors such as the hydrodynamic radius of the drug, radii of the vascular pores, fraction of large pores, and hydraulic conductivity of the vascular endothelium influence this exchange.

- **Transport into lymph vessels:** The drug is transported into lymph vessels through convection, which is modeled using reflection coefficients.
- **Uptake into endothelial cells and recycling:** The drug is taken up into endothelial cells via pinocytosis and can recycle back into the plasma. By default, the distribution model for large molecules in PK-Sim assumes that there is no exchange between interstitial space and endosomes.
- **Degradation in endosomes:** The free drug undergoes degradation in the endosomes of endothelial cells. This degradation process has a predefined rate constant in PK-Sim.
- **Protection from endosomal degradation:** Proteins which contain an Fc part (such as mAbs and Fc fusion proteins) can be protected from endosomal degradation by binding to FcRn. This binding is characterized by the dissociation rate constant $k_{\text{off},\text{FcRn}}$, the association rate constant $k_{\text{on},\text{FcRn}}$, and the equilibrium dissociation constant $K_{\text{D},\text{FcRn}} = k_{\text{off},\text{FcRn}} / k_{\text{on},\text{FcRn}}$.

The FcRn sub-model of OSP suite is based on the model published earlier by Garg and Balthasar (Garg and Balthasar, 2007). According to the model, the drug enters the vascular endothelium, which is represented by a compartment for protein degradation and recycling, at a first-order uptake rate. Drug that binds to FcRn within the endosomal space is protected from degradation and recycled at a first-order rate to the plasma space (for simplicity, PK-Sim does not consider recycling to the interstitial space by default). Unbound drug, however, is cleared from the endosomal space, again at a first-order rate.

Furthermore, the PK-Sim model structure incorporates the representation of endogenous IgG, which competes with the drug for the FcRn receptor. Thus, drug elimination is influenced by the general endosomal uptake-, recycling- and

degradation rate constants, the affinity of the drug to FcRn, the endosomal concentration of the FcRn receptor, as well as the endosomal concentration of endogenous IgG. In contrast to the model of Garg and Balthasar, the OSP suite model allows for different affinities to be specified for the drug and the endogenous IgG, as drug FcRn-binding is explicitly represented (Open Systems Pharmacology Suite Manual Version 7.4, 2018).

To account for TMDD processes in the large molecule model of OSP suite, the described model structure can be extended within the OSP modeling software tool MoBi. This can be done by adding reactions accounting for target turnover, reversible target-binding and drug-target-complex degradation (Open Systems Pharmacology Suite Manual Version 7.4, 2018).

Ocular pharmacokinetics of large molecules

While whole-body PBPK models are constantly advancing, with several softwares adding new information of various organs, the eye is still poorly investigated in terms of a mathematical representation and not yet included in standard software like OSP suite. An understanding of the PK processes of ocular drugs is crucial to advance such a development. Protein therapeutics or their fragments are widely used for treating diseases of the retina (Amo et al., 2017). They can be administered via an ivt injection into the eye (Peyman et al., 2009). For large molecule drugs, not all the structures of the eye are accessible. After ivt administration, a large molecule drug can diffuse through the vitreous humor. Experiments on vitreal diffusion of molecules and nanoparticles using particle tracking methods and confocal microscopy suggest that the mesh size in the bovine vitreous is in the range of 500 nm (Peeters et al., 2005; Xu et al., 2013). Thus, the vitreous is not a barrier for diffusion (Turunen et al., 2016), allowing the mobility of molecules in the size of mAbs or their fragments (Li et al., 2011). However, positive charge can have an impact on diffusion due to electrostatic interactions with the negatively charged vitreous polymer network (Amo et al., 2017). Also, a cationic charge density on the hydrophobic regions of a molecule might affect vitreal diffusion, but this has not been clarified.

Drugs can be excreted from the vitreous via two routes, the anterior or the posterior one. The anterior route involves drug diffusion through the vitreous towards the aqueous chamber. From there it can be eliminated with the aqueous humor via the Schlemm's canal. This route is dependent on the drug diffusion through the vitreous humor, which correlates with the hydrodynamic molecular radius of a compound (Shatz et al., 2016). Via the posterior route, a drug is cleared towards the retina. The retinal tissue constitution is more complex than other ocular structures (see Figure 7) and drug disposition at the back of the eye is not well understood, yet.

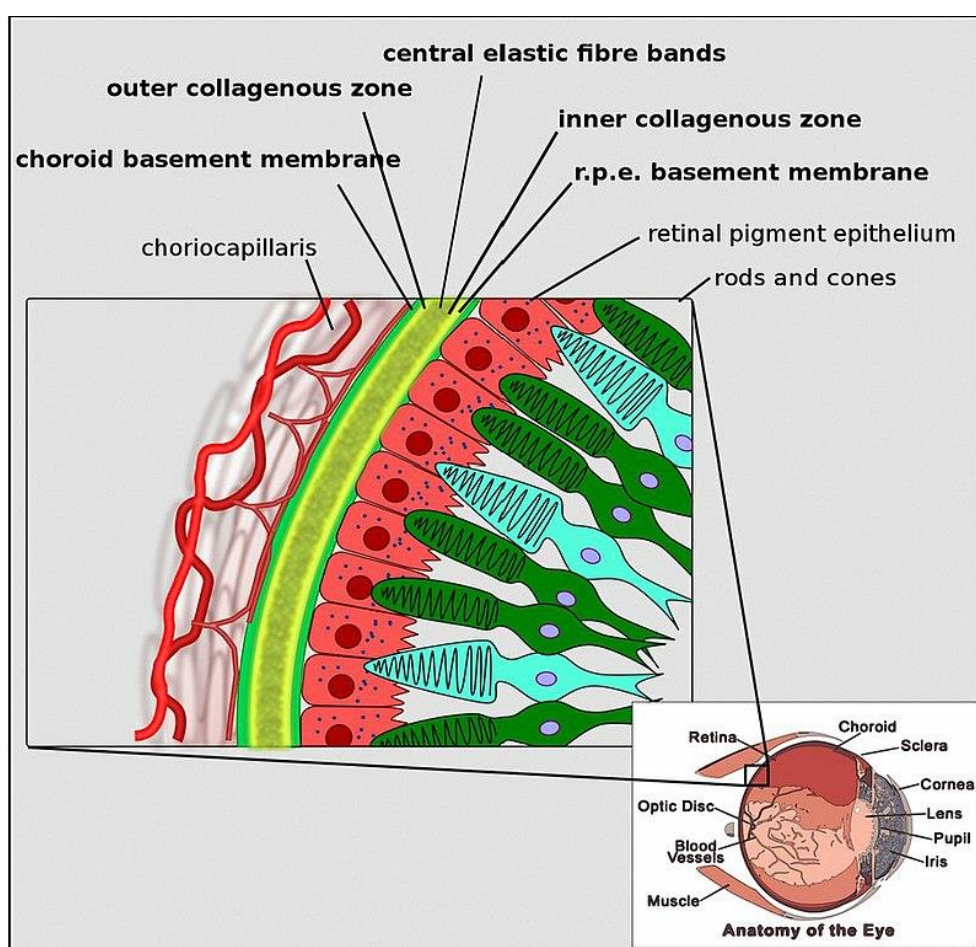


Figure 7. Posterior eye segments. From anterior to posterior: retinal cells (rods and cones), retinal pigment epithelium, Bruchs' membrane (containing retinal pigment epithelium (r.p.e.) basement membrane, inner collagenous zone, central elastic fibre bands, outer collagenous zone, choroid basement membrane), choriocapillaris. Figure available at Wikimedia Commons (downloaded 2024-06-04,

https://commons.wikimedia.org/wiki/File:Retinal_pigment_epithelium.jpg, author: MethylC5).

The various cells in the retina may not be accessible to large molecules, as their size typically prevents them from penetrating cell membranes. Different barriers play a role in retinal drug disposition. First of all, it is assumed that retinal blood vessels, located in the inner retinal layers, are impermeable to large molecules due to their dense endothelial connections (Amo et al., 2017). Furthermore, the ILM represents a barrier towards the vitreous body. Its structure of glycosaminoglycans and collagen does not stop small molecules but may limit the diffusion of large molecule drugs. Studies suggest that there is a limitation of molecular size for the penetration of the ILM, but many large molecules can pass relatively unrestricted (Amo et al., 2017). The permeability of the posterior retinal barrier, the RPE, is not well investigated and it is even questioned if large molecules can penetrate the RPE at all (Amo et al., 2017). However, in-vitro cell experiments showed that bevacizumab is able to penetrate a porcine RPE cell layer (Terasaki et al., 2015). Furthermore, ex-vivo experiments suggested that the RPE is permeable to bevacizumab (Ramsay et al., 2019). Compared to the RPE, the adjacent Bruch's membrane and choroid exhibit a higher permeability, which is why they are not thought to limit drug permeation (Miller and Edelman, 1990; Pitkänen et al., 2005). The choroidal blood vessels covering the Bruchs' membrane, are highly fenestrated (Bill et al. 1980) and thus, permeable for large molecules (Amo et al., 2017). The specific contribution of each retinal segment layer in ocular drug disposition remains unclear, creating a research objective for ocular PK models.

Published ocular models

Several ocular PK models have been developed so far, each with different approaches and considerations. Schmitt et al. (Schmitt, 2017) utilized physics-based equations to predict ocular PK based on the molecular weight of a compound. Rimpelä et al. (Rimpelä et al., 2021) developed a mechanistic model for small molecules, taking into

account the diffusion rate from vitreous to aqueous and the permeability across posterior segment tissues. Both, Schmitt and Rimpelä, validated their models using vitreal half-life values. Hutton-Smith et al. (Hutton-Smith et al., 2017; Hutton-Smith et al., 2018) introduced 3-compartment models for macromolecules in rabbit and human, considering vitreous to aqueous diffusion and posterior segment permeabilities. In their model, drug that reaches the aqueous chamber is eliminated via Schlemm's canal. Drug permeating the inner limiting membrane (ILM), reaches the retina and exits through the retinal pigment epithelium (RPE). Recently, Naware et al. (Naware et al., 2023) and Bussing and Shah (Bussing and Shah, 2020) published ocular physiologically based pharmacokinetic (PBPK) models for large molecules in rabbit, monkey, and human. They incorporated the posterior eye permeabilities estimated by Hutton-Smith et al. (Hutton-Smith et al., 2017).

Developing mechanistic ocular models presents challenges due to limited knowledge of various physiological parameters. Firstly, the elimination rate from vitreous to aqueous depends on diffusion, which in turn depends on the molecular weight (MW) of the drug. A linear relationship between vitreal diffusion time and vitreal half-life was found by Caruso et al., but this relationship is not consistent across different species (Caruso et al., 2020). Secondly, uncertainties arise from the permeability of the ILM (pILM) and the permeability of the RPE (pRPE). Hutton-Smith et al. (Hutton-Smith et al., 2017) compared their estimated pILM and pRPE values with the molecular weight of compounds, but they found that the relationship is not linear and therefore possibly not as straightforward as for the vitreal diffusion rate. Literature suggests that posterior segment permeability may depend not only on MW but also on the charge of a molecule (Peynshaert et al., 2018). While for pILM there is no in-vitro data available to compare with the estimations of Hutton-Smith et al. (Hutton-Smith et al., 2017), estimated values for pRPE deviate significantly from in-vitro measurements (Hutton-Smith et al., 2017; Terasaki et al., 2015; Ramsay et al., 2019). Furthermore, accurately determining the effective retinal volume is challenging, as the entry of large molecule drugs into cells is typically limited. The retina consists to a large part of cells, and reported retinal volumes usually consider the whole retina, including the cellular, blood vessel and interstitial fractions. Consequently, accurately describing large molecule drug concentrations within the retina is difficult.

In addition to the challenges in mechanistic ocular modeling, most current models treat the eye as an isolated organ. This limitation becomes particularly relevant in human studies where eye sampling is rare, and model development often relies heavily on plasma data. Systemic toxicological effects observed after intravitreal administration emphasize the importance of understanding ocular drug PK within a systemic context.

Outline

This dissertation aims to improve our mechanistic understanding of the ocular PK of large molecule drugs in different species. Using PK modeling as an effective tool to investigate intravitreally administered drugs, important objectives were to improve our knowledge about the permeability of ocular tissue barriers, and to describe drug exposure at the target site without retinal sampling available. The developed models should consider physiological differences between animal species. Putting the eye within a systemic context by taking into account not only ocular PK but also systemic PK of a drug will improve our understanding of the blood-ocular barrier as well as enable future predictions based on sparse data (or plasma data only).

To improve ocular modeling across different species, and to bring the developed models into a context of whole-body PK, we aimed

- to develop a precise model structure for ocular PK,
- to understand inter-species differences of systemic large molecule PK,
- and to explore cross-species translation of ocular PK.

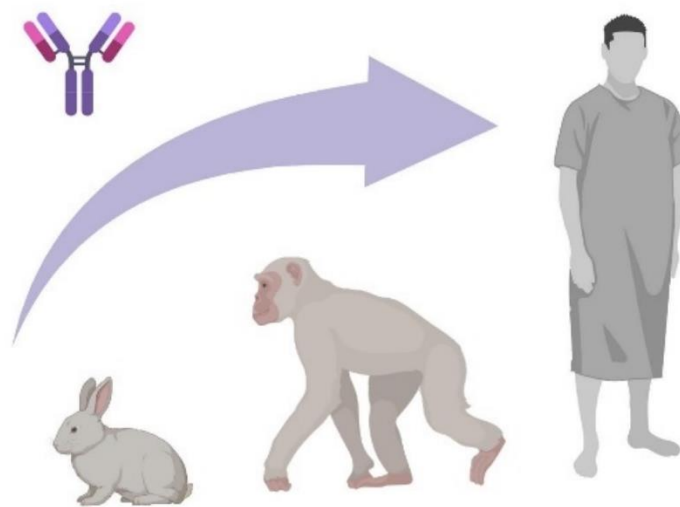
Chapter 2 illustrates a case study for the translation of a large molecule PBPK model between rabbit, monkey, and human. Using the modeling platform OSP suite, PBPK models were built, and the relevant physiological parameters for drug elimination and target-binding were compared between species.

Chapter 3 explores ocular PK in rabbits (the species with most data available), developing an extended ocular model structure, including injected eye, systemic compartments and fellow eye. The work introduces refined model parameters and offers a detailed model structure based on data that was not analyzed previously for ocular model development.

Chapter 4 describes a cross-species translation of an extended ocular PK model for ranibizumab, allowing for identification of major differences in ocular PK between preclinical species and humans.

Together, the results of this work improve our understanding of ocular drug PK, as well as cross-species translation of large molecule models. The translational efalizumab PBPK models help to understand cross-species differences of systemic large molecule PK. The extended ocular rabbit PK model incorporating the fellow eye contributes to a more detailed understanding of ocular PK. Finally, the translational ocular PK models for ranibizumab help to explore cross-species translation of ocular PK.

Chapter 2: PBPK-based translation from preclinical species to humans for the full-size IgG therapeutic efalizumab



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Contributions:

M. Franz developed the model, performed the simulations, analyzed the data, created the figures, and wrote the chapter. N. Hanke, R. Jairam and L. Kuepfer discussed the data and reviewed the chapter.

Figures of this chapter were partially created with BioRender.com.

Abstract

Animal models are crucial in pharmaceutical research and development, as they guide the planning and design of subsequent clinical studies. Ensuring confidence and accuracy in first-in-human dose estimates requires assessing how well animal studies translate to human conditions. This is particularly important for large molecules, where it is mandatory to evaluate the cross-species translatability of key parameters such as neonatal fragment crystallizable receptor (FcRn) binding and target-mediated drug disposition (TMDD), which significantly affect protein distribution and clearance.

In this study, plasma pharmacokinetic data for the therapeutic protein efalizumab were sourced from literature, and physiologically based pharmacokinetic (PBPK) models were developed for three species: rabbits, non-human primates (NHP), and humans. The models for NHP and humans included parameters for target-binding, and the study explored whether assumptions of translatable target turnover and similar target binding between these two species held true.

The developed PBPK models successfully characterized efalizumab's concentration-time profiles across various intravenous doses in all three species. For NHP and human models, shared parameters for target turnover and internalization of drug-target-complexes were established, along with similar target-binding characteristics. However, our analysis indicated that translating FcRn affinity parameters between species is not feasible, while TMDD-related parameters could be transferred from NHP to humans. These findings emphasize the importance of species-specific FcRn affinity values to accurately capture the respective concentration-time profiles.

To strengthen and validate these conclusions, further pharmacokinetic data on efalizumab across different doses would be beneficial. Additionally, incorporating information on target expression and internalization rates could enable the creation of robust human PBPK models with reduced reliance on animal testing. Using a free and open-source PBPK modeling platform (Open Systems Pharmacology Suite, <http://www.open-systems-pharmacology.org>), this study demonstrated that cross-

species comparisons of therapeutic proteins like efalizumab can enhance the predictability of first-in-human doses, potentially guiding future translational research.

Introduction

Animal models are fundamental in pharmaceutical research and development, providing critical insights into pharmacokinetics, safety, and efficacy before advancing to first-in-human clinical studies. However, predicting human outcomes based on preclinical species remains a significant challenge. Traditional methods like allometric scaling often fail to produce reliable predictions (Ward et al., 1999; Mahmood, 2009; Jairam et al., 2018).

PBPK modeling offers a more robust and mechanistic approach to interspecies scaling (Kuepfer et al., 2016). PBPK modeling allows for a detailed representation and translation of drug absorption and disposition, taking into account the drug's physicochemical properties and the anatomical and physiological characteristics of each species. While drug-specific parameters require prior knowledge or assumptions, PBPK software tools like PK-Sim provide built-in information on whole-body physiology across different species (Willmann et al., 2005; Kuepfer et al., 2016; Thiel et al., 2017).

Previous efforts have aimed to develop PBPK models capable of cross-species translation, primarily focusing on small molecules, as summarized by Thiel et al. (Thiel et al., 2015). However, fewer studies have explored this approach for large molecules. Shah and Betts (2012) constructed PBPK models for intravenously administered mAbs in mice, rats, monkeys, and humans for similar but not identical compounds, likely due to limited data availability. Sepp et al. conducted two studies successfully translating mAb PBPK models from rodents to primates for the same compound (Sepp et al., 2020). More recently, Pasquiers et al. (Pasquiers et al., 2023) presented a cross-species PBPK model for bevacizumab, translating from monkeys to humans.

Rabbits are frequently used in animal testing, especially to develop treatments for ocular diseases such as age-related macular degeneration and diabetic retinopathy. Translating findings from rabbits to NHP is common practice. Naware et al. recently developed an ocular PBPK model for rabbits, monkeys, and humans following systemic and intravitreal administration (Naware et al., 2023). However, the data sets used had linear pharmacokinetics (PK), minimizing target-binding effects, which were not investigated. This is significant because target-binding effects in rabbits can be absent or weaker compared to humans, as is the case with bevacizumab (EMA, 2005).

In this study, we focused on efalizumab, a humanized immunoglobulin G-1 (IgG1) monoclonal antibody previously used in the treatment of psoriasis (Mortensen et al., 2005a). Efalizumab targets the CD11a subunit of leukocyte function-associated antigen 1 (LFA-1), an adhesion receptor expressed on leukocytes, and was developed as an immunosuppressant inhibiting lymphocyte activation. Its pharmacokinetics have been studied following intravenous administration in rabbits, NHPs, and human psoriasis patients (Bauer et al., 1999; Gottlieb et al., 2000, 2002; Ng et al., 2005; Mould and Green, 2010; Chetty et al., 2015). In humans, efalizumab exhibits dose-dependent clearance, with a plasma half-life of 5.3 days at 10 mg/kg, which decreases to 0.4 days at 0.1 mg/kg due to faster clearance at lower doses (Bauer et al., 1999; Gottlieb et al., 2000b). This dose-dependent behavior is due to target-mediated drug disposition (TMDD), which creates characteristic concentration-time profiles comprising four phases: an initial rapid decline due to target-binding, a linear phase dominated by free drug degradation, a transition phase as target saturation decreases, and a final terminal phase where elimination occurs primarily via clearance of the drug-target-complex when target availability is no longer rate limiting (Peletier and Gabrielsson, 2012).

Non-linear PK has also been observed in chimpanzees, which aligns with efalizumab's binding affinity to CD11a in this species (Bauer et al., 1999). In contrast, efalizumab is not cross-reactive (no target binding) in rabbits, resulting in linear PK (Mortensen et al., 2005b; Mould and Green, 2010). The non-saturable component of efalizumab clearance occurs through endosomal protein degradation. For antibodies with an Fc region, binding to the neonatal fragment crystallizable

receptor (FcRn) can prevent endosomal clearance. While efalizumab does not bind CD11a in rabbits, FcRn-binding remains relevant, as it has been observed in-vitro for other humanized IgG antibodies (Szikora et al., 2017). Figure 8 summarizes the primary processes influencing efalizumab PK, which are crucial to understand for cross-species translation among the three species studied.

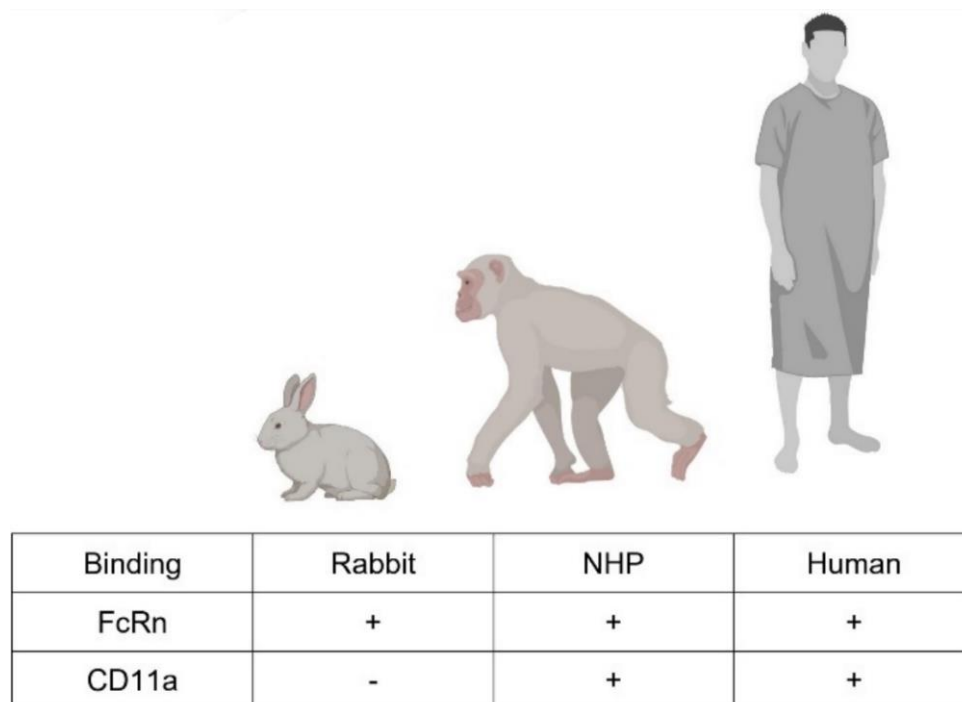


Figure 8. Efalizumab cross-species translation between rabbit, monkey, and human. Two mechanisms that highly influence the PK of large IgG molecules are FcRn-binding and target-binding. Efalizumab binds to rabbit, non-human primate (NHP) and human FcRn. Target-binding to CD11a is only present in NHP and human, but not in rabbit (Bauer et al., 1999; Mortensen et al., 2005; Mould and Green, 2010).

In the current study, we investigated the translatability of large molecule PBPK models between rabbit, NHP and human. The therapeutic protein efalizumab was selected for this case-study, as time-resolved plasma PK data after intravenous administration were available in the literature for all three species. A full TMDD model was used to build models with physiologically interpretable parameters that can be evaluated

against experimental values from literature (see Results). The assumptions of comparable target-binding kinetics, and the same target turnover and drug-target-complex internalization, but different FcRn-binding kinetics between species was explored, to gain insights into how these parameters might be translated for first in human predictions.

Results

The goal of the current study was to investigate the translatability of large molecule PBPK models between rabbit, NHP and human. We selected the therapeutic protein efalizumab for our case-study, as time-resolved data of plasma samples after intravenous administration were available in the literature for all three species. The developed models contain physiologically interpretable parameters that can be evaluated against experimental values and compared between species. In particular, parameters related to FcRn affinity, target-binding, target turnover and drug-target-complex internalization were explored. As a first step, we developed and evaluated the rabbit model, before moving to a more complex model structure for NHP and human.

Model structure

PK-Sim's PBPK model structure for large molecules was used as a basis for model development (Niederalt et al., 2018). It includes the typical mechanisms of large molecule disposition which is governed by the following physiological and biochemical processes:

- Transcapillary exchange of the drug between plasma and interstitial space, described by the two-pore formalism (Rippe and Haraldsson, 1987; Rippe and Haraldsson, 1994). The drug passes through small and large vascular pores by convection and diffusion. This is influenced by the hydrodynamic radius of the

drug, the radii of the vascular pores, the fraction of large pores, and the hydraulic conductivity of the vascular endothelium.

- Transport into lymph vessels by convection.
- Uptake of the drug into endothelial cells via pinocytosis.
- Degradation of the free drug in endosomes of endothelial cells (fixed degradation rate constant in PK-Sim).
- Protection from endosomal degradation by binding of drug to FcRn, characterized by the dissociation rate constant $k_{off,FcRn}$, the association rate constant $k_{on,FcRn}$ and the equilibrium dissociation constant $K_{D,FcRn} = k_{off,FcRn} / k_{on,FcRn}$.

The OSP modeling software tool MoBi was used for an extension of the NHP and human PBPK model structures to include the TMDD processes given below:

- Target synthesis and internalization, modeled via the steady state target concentration (T_{ss}) and a target internalization rate constant ($k_{int,T}$).
- Reversible binding of drug to free target (characterized by the rate constants k_{on} and k_{off}), forming a drug-target-complex.
- Internalization of the drug-target-complex, modeled by the rate constant $k_{int,C}$.

The following equations were used to account for these processes:

$$\frac{dD}{dt} = -k_{on} \cdot D \cdot T + k_{off} \cdot C$$

$$\frac{dT}{dt} = k_{int,T} \cdot (T_{ss} - T) - k_{on} \cdot D \cdot T + k_{off} \cdot C$$

$$\frac{dC}{dt} = k_{on} \cdot D \cdot T - k_{off} \cdot C - k_{int,C} \cdot C$$

with C: drug-target-complex, D: drug, k_{off} : dissociation rate constant, k_{on} : association rate constant ($k_{on} = k_{off} / K_D$, using the equilibrium dissociation constant K_D), $k_{int,C}$:

internalization rate constant of the drug-target-complex, $k_{int,T}$: internalization rate constant of the target, T: target, and T_{ss} : steady state target concentration.

The equations given above represent a full TMDD model, i.e., they contain physiologically interpretable rate constants, rather than lumping them together in one parameter (Peletier and Gabrielsson, 2012). This allows for a comparison of single parameters between species. More precisely, it enabled to explore if values for $k_{int,C}$, $k_{int,T}$, k_{off} and T_{ss} can be translated between NHP and human. The relevant processes for target-binding and FcRn-binding are schematically displayed in Figure 9. This is a simplified representation of the processes incorporated to extend the PBPK model structures of NHP and human; the complete implementation has been published previously by Niederalt et al. (Niederalt et al., 2018).

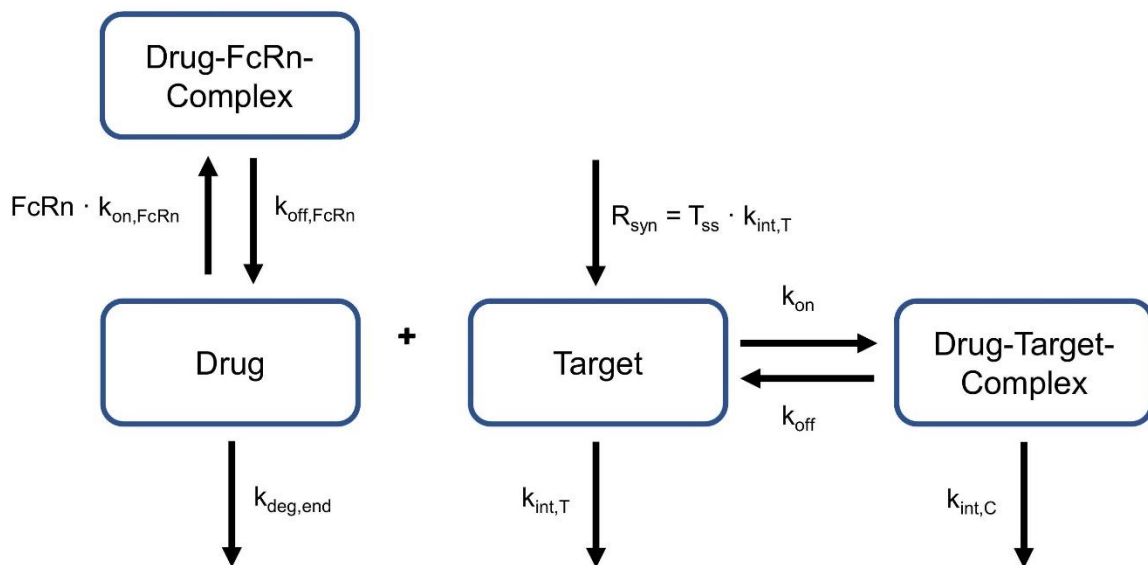


Figure 9. Relevant reactions for target- and FcRn-binding. Drug can bind to and dissociate from target and FcRn. Target is produced continuously. Target and drug-target-complex are internalized with different rates. Target turnover and target-binding is only applicable to NHP and humans, not to rabbits. FcRn: neonatal Fc Receptor, $k_{deg,end}$: degradation rate constant in endosomes, $k_{int,C}$: internalization rate of the drug-target-complex, $k_{int,T}$: internalization rate of the target, k_{off} : dissociation rate constant

of the drug-target-complex, $k_{off,FcRn}$: dissociation rate constant of the drug-FcRn-complex, k_{on} : association rate constant of the drug-target-complex, $k_{on,FcRn}$: association rate constant of the drug-FcRn-complex, R_{syn} : zero-order synthesis rate of the target, T_{ss} : target concentration in steady state.

Model parameters

The hydrodynamic radius of efalizumab was assumed to be identical between species, as it is a physico-chemical parameter that depends on the drug only. Thus, the same value of 3.51 nm was used for all species. It was calculated with the formula (Hutton-Smith et al., 2016):

$$R_{hyd} = \left(\frac{3vMW}{4\pi N_A} \right)^{1/3}$$

with MW: molecular weight, N_A : Avogadro's number, and v : partial specific volume of protein, taken as 0.73 cm³/g.

$K_{D,FcRn}$ of the human model was fixed to the value provided by the OSP suite (Niederalt et al., 2018). It was shown previously that FcRn-binding kinetics differ between species (Abdiche et al., 2015). Thus, $K_{D,FcRn}$ for the rabbit and NHP model were not taken from the human model, but estimated. The association rate constant $k_{on,FcRn}$ as well as the degradation rate constant in endosomes $k_{deg,end}$ were taken as provided in the OSP suite.

Furthermore, the CD11a expression reference concentration in the organ with maximal target expression (only relevant for NHP and human) was assumed to be the same in NHP and human. The OSP suite includes publicly available data of relative gene expression across all organs and tissues in the whole-body model, which can be added to a PBPK model (Meyer et al., 2012). The relative expression profile of CD11a from the human gene expression "Expressed Sequence Tags (EST)" database was

integrated into both, the NHP and human model. CD11a was assumed to be expressed in plasma and interstitial space, according to the location of leucocytes.

Efalizumab binding to CD11a was studied in-vitro for NHP and human. Bauer et al. reported comparable K_D values for NHP and human of 0.99 nM and 0.77 nM, respectively (Bauer et al., 1999). K_D values were fixed to these literature values in the NHP and human models. As no literature was found on the dissociation rate constants, the same value for k_{off} was assumed for both models. Target-binding of mAbs is generally thought to be similar between NHPs and humans due to the high sequence homology (Germovsek et al., 2021). Furthermore, the internalization processes of CD11a and drug-bound CD11a, and with that, the values for $k_{int,T}$ and $k_{int,C}$, respectively, were assumed to be the same in NHPs and humans.

CD11a reference concentration, k_{off} , $k_{int,T}$ and $k_{int,C}$ were estimated. During the estimation procedure, $k_{int,C}$ was restricted to differ from $k_{int,T}$ by a scaling factor between 0.1 and 10. This allowed the drug-induced change in the internalization rate to remain in a similar range as observed by Bauer et al. and Ng et al., who estimated a maximum of 3- (Bauer et al., 1999) or 5-times (Ng et al., 2005) increased internalization rate of CD11a induced by efalizumab-binding in humans. For the human model, infusion time was estimated with boundaries between 60 and 180 min, as the exact values were not provided in the literature source.

Modeling outcome

First, PK data of efalizumab in rabbits were considered (Mould and Green, 2010). Concentration–time profiles following intravenous administration of 2 mg/kg efalizumab in rabbits could be adequately described by the model without target-binding (Figure 10), even though only one parameter ($K_{D,FcRn}$) was allowed to vary during the estimation process.

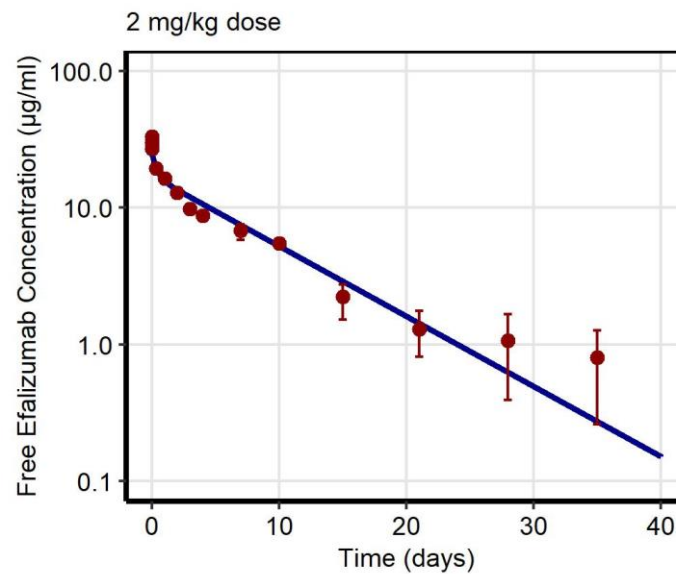


Figure 10. Free efalizumab concentrations over time in rabbit. Line: Simulation, points: observed data (Mould and Green, 2010).

After demonstrating the applicability of the structure for the rabbit model, we moved on to the NHP and human models, where different FcRn-binding kinetics and additional TMDD processes were expected. The models with target-binding were able to describe the concentration–time profiles of efalizumab in NHP for 8 mg/kg intravenous administration (Figure 11) and in human for 0.1, 0.3, 1.0, 3.0 and 10 mg/kg intravenous administration (Figure 12). Notably, the model structure could well capture the PK behavior for doses over 2 orders of magnitude.

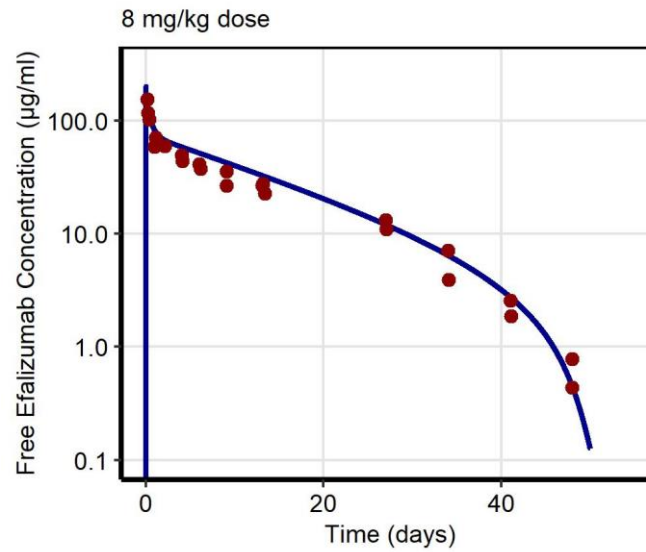


Figure 11. Free efalizumab concentrations over time in NHP. Line: Simulation, points: observed data (Bauer et al., 1999).

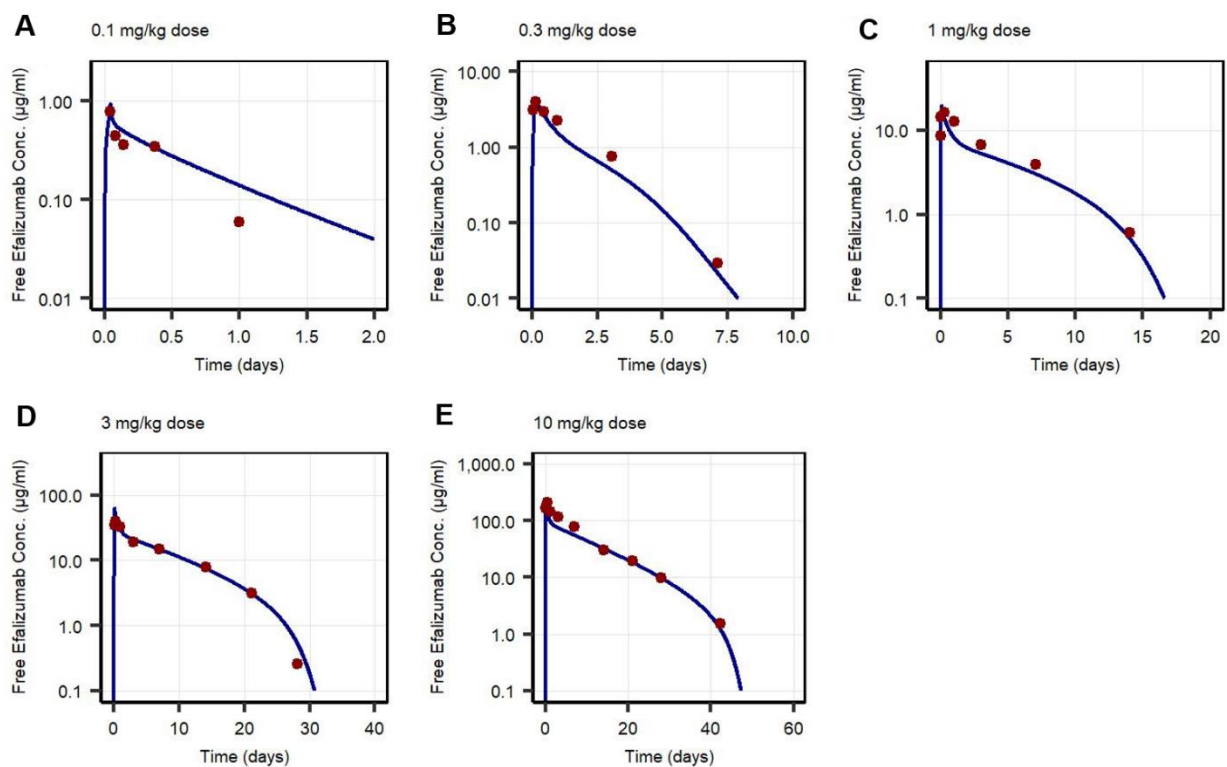


Figure 12. Free efalizumab concentrations over time in human following intravenous administration of (A) 0.1 mg/kg, (B) 0.3 mg/kg, (C) 1.0 mg/kg, (D) 3.0 mg/kg, (E) 10 mg/kg. Lines: Simulations, points: observed data (Bauer et al., 1999).

Table 1 summarizes the estimated and fixed parameters of the three developed PBPK models. MW, human $K_{D,FcRn}$, K_D , and association rate constant of efalizumab binding to FcRn ($k_{ass,FcRn}$) were fixed to literature or PK-Sim default values (Bauer et al., 1999; Boehncke, 2007; Niederalt et al., 2018). Rabbit and NHP $K_{D,FcRn}$ were estimated. CD11a reference concentration, k_{off} , $k_{int,T}$ and $k_{int,C}$ were estimated for the NHP and human models, assuming that there is no inter-species difference in target-binding kinetics. The difference between $k_{int,C}$ and $k_{int,T}$ was restricted to a maximum factor of 10 (see **Model parameters**). Estimated infusion time for the human administrations was 180 min for all doses, except for the lowest dose of 0.1 mg/kg (60 min).

Table 1. Parameters of efalizumab PBPK models.

Parameter	Rabbit	NHP	Human	Source	Description
MW [kDa]	149	149	149	(Boehncke, 2007)	Molecular weight
$K_{D,FcRn}$ [μ M]	6.51	0.49	0.63	Rabbit and NHP: estimated, human: Niederalt et al., 2018	Equilibrium dissociation constant of efalizumab binding to FcRn
CD11a reference concentration [μ M]	-	0.12*	0.12*	Estimated	Concentration in organ with maximal expression (= gonads)
$k_{int,T}$ [h^{-1}]	-	0.005*	0.005*	Estimated	Internalization rate constant of CD11a

$k_{int,C}$ [h^{-1}]	-	0.05*	0.05*	Estimated	Internalization rate constant of the efalizumab-CD11a-complex
K_D [nM]	-	0.99**	0.77**	(Bauer et al., 1999)	Equilibrium dissociation constant of efalizumab binding to CD11a
k_{off} [h^{-1}]	-	0.33*	0.33*	Estimated	Dissociation rate constant of the efalizumab-CD11a-complex
R_{hyd} [nm]	3.51	3.51	3.51	Calculated	Hydrodynamic radius
$k_{ass,FcRn}$ [$\mu M^{-1} \cdot min^{-1}$]	0.87	0.87	0.87	PK-Sim default	Association rate constant of efalizumab binding to FcRn

*same value assumed for NHP and human

**in-vitro measurement

Figure 13 shows the results of the local sensitivity analyses for all three models (for the human model the 3 mg/kg dose is presented; results for the other doses can be

found in the Supplementary Material). Parameters related to target turnover, target binding and FcRn binding were selected for the analysis in order to quantify their relevance for cross-species translation. This selection included all estimated model parameters, as well as parameters which were assumed to have a strong influence on PK. All models show highest sensitivity to the value of $K_{D,FcRn}$. This suggests that rescue from endosomal clearance is the most relevant process determining efalizumab PK after administration of the investigated doses. For the human and NHP models, CD11a reference concentration and $k_{int,T}$ are moderately impactful parameters, while target-binding parameter values and $k_{int,C}$ have no significant impact on the predicted AUC extrapolated to infinity.

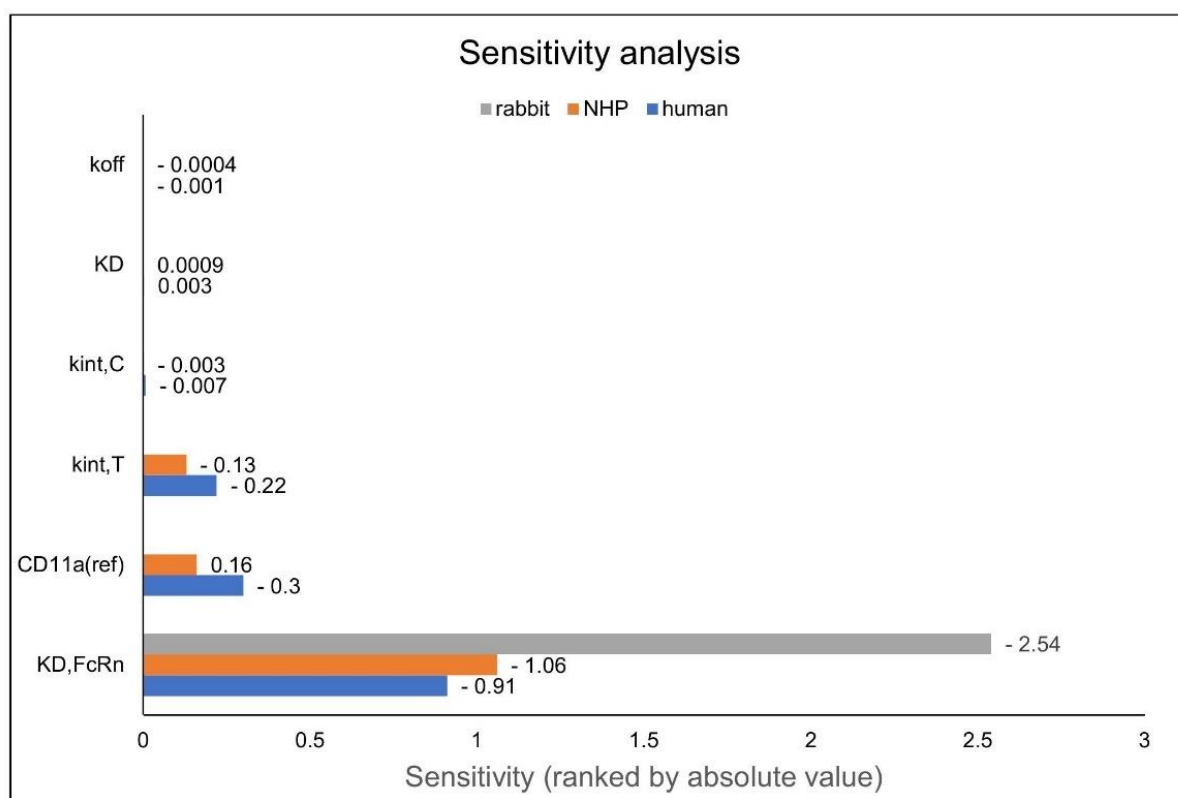


Figure 13. Local sensitivity analysis results of the rabbit, NHP and human models. Sensitivity of the models to single parameters, ranked by their impact on the predicted value of AUC extrapolated to infinity. CD11a(ref): CD11a reference concentration, K_D : equilibrium dissociation constant of the drug-target-complex, $K_{D,FcRn}$: equilibrium dissociation constant of the drug-FcRn-complex, $k_{int,C}$: internalization rate of the drug-

target-complex, $k_{int,T}$: internalization rate of the target, k_{off} : dissociation rate constant of the drug-target-complex, NHP: non-human primate.

Discussion

Mechanistic whole-body PBPK models of efalizumab were developed in rabbits, non-human primates, and humans. The models effectively describe the available preclinical and clinical data across the three species, enabling a mechanistic comparison of the physiological processes underlying the pharmacokinetics of efalizumab in rabbits, NHP, and humans. The use of a qualified PBPK platform facilitates the investigation of cross-species differences and supports future application of the developed models. The new models were used to analyze the differences in specific parameters, reflecting distinct physiological processes, and to test the assumption of similar target binding and translatable target internalization in NHP and human. Consequently, this study offers valuable insights into the inter-species translatability of large-molecule PBPK models.

The newly developed models successfully captured concentration–time profiles following intravenous administration, with only minor deviations from observed data. For the rabbit model, the predictions were within the error margins, except for two data points beyond 28 days post-dose, which were underpredicted. This underestimation could be due to experimental limitations as the drug concentrations approached the assay's lower limit of quantification (0.025 µg/ml). However, the observed data in question is still considerably higher than this threshold. Alternatively, peripheral tissue binding and subsequent redistribution of efalizumab could explain this discrepancy, although no supporting evidence could be found in literature. Following the principle of parsimony, the model structure was kept basic and without implementation of any hypotheses to better capture these last two data points. Additionally, the original data source (Mould and Green, 2010) claims that the PK profile is linear.

For the human model, the observed concentration 1 day after the 0.1 mg/kg dose was overpredicted. This could be attributed to the internalization rate which highly

influences the last phase of a classical TMDD profile. $k_{int,T}$ and $k_{int,C}$ were restricted to a certain range (maximum 10-fold difference) during parameter identification.

Although relaxing this restriction would result in a better fit for the 0.1 mg/kg dose profile, it would be less consistent with the models proposed in literature. Bauer et al. (Bauer et al., 1999) estimated a maximum 3-fold increase of internalization rate induced by efalizumab binding to the target for human and a maximum 4.5-fold increase for monkey. According to the model of Ng et al. (Ng et al., 2005), internalization is maximum 5-times increased upon efalizumab binding in humans. $K_{D,FcRn}$ for human was fixed to the value of 0.63 μ M provided in the OSP suite (Niederalt et al. 2018). The FcRn affinity for efalizumab measured in-vitro is lower, with a $K_{D,FcRn}$ of 1.8 μ M (Chung et al., 2019). Also, Suzuki et al. (Suzuki et al., 2010) predicted a lower affinity (2.69 μ M) with a linear regression model.

The estimated $K_{D,FcRn}$ for NHP was 0.49 μ M, which corresponds to a 22% higher affinity compared to binding to the human FcRn. It was shown in in-vitro experiments that cynomolgus monkey FcRn binds to humanized IgG1 with a higher affinity than human FcRn. A 50 - 60% higher affinity was reported from surface plasmon resonance biosensor assays (Dall'Acqua et al., 2006; Abdiche et al., 2015). Other studies also observed higher affinity in NHP compared to human using a biolayer interferometry assay. They report a 60 - 75% higher affinity of humanized IgG1 for cynomolgus monkey compared to human FcRn (Deng et al., 2010; Neuber et al., 2014). As intravenous data from chimpanzees and not cynomolgus monkeys were used for this model, the differences in affinity may not be fully translatable, however there might be a similar trend towards higher FcRn affinity for humanized IgG1.

The estimated $K_{D,FcRn}$ for the rabbit model was 6.51 μ M, which is about 10-fold higher than for human. In-vitro experiments have shown that rabbit FcRn has a lower affinity for humanized IgG1. However, the study reports only a 2-fold higher $K_{D,FcRn}$ for rabbit compared to human (Szikora et al., 2017).

Even though these results are in line with the trends in literature, the interpretation has to be treated with caution. The estimation of $K_{D,FcRn}$ depends on a number of parameters, like the endosomal FcRn concentration and $k_{on,FcRn}$, as well as the endogenous IgG concentration and related binding kinetics. For these parameters the default values of PK-Sim were used and potential errors in these default parameter

values are passed on to the $K_{D,FcRn}$ estimates. Especially for the rabbit model, the default values are not validated sufficiently: for human and monkey, PK-Sim incorporates species specific values for FcRn concentration, endogenous IgG concentration and the affinity of endogenous IgG to FcRn, while the rabbit model uses only “reasonable values” (Open Systems Pharmacology Suite Manual Version 7.4, 2018).

The NHP and human models suggest an internalization rate of CD11a of 0.005 h^{-1} . This value is lower than predicted by the models of Bauer et al. and Ng et al. which were around 0.02 h^{-1} (Bauer et al., 1999; Ng et al., 2005).

The internalization rate of the efalizumab-CD11a-complex was estimated to be 0.05 h^{-1} . Coffey et al. (Coffey et al., 2004) measured the internalization of antibody-linked efalizumab into human blood T cells in the presence of concanamycin A (to inhibit cellular clearance). They observed that 48% of the drug was internalized by the cells after 24 h. Assuming a linear uptake rate, this corresponds to an internalization rate of 0.02 h^{-1} . Thus, the estimated value of $k_{int,C}$ has the same dimension as the in-vitro result. Comparing the estimated $k_{int,T}$ and $k_{int,C}$, CD11a internalization is 10-fold increased upon efalizumab binding. Bauer et al. and Ng et al. only estimate a 3- to 5-fold increased internalization upon efalizumab binding (Bauer et al., 1999; Ng et al., 2005).

It is worth noticing however, that the monkey model structure utilized in PK-Sim was initially designed for a lightweight macaque monkey (Willmann et al., 2007). As in PBPK the body weight is the sum of all organ weights, it is difficult to apply allometric scaling. When adjusting the body weight to match that of a chimpanzee, a relatively straightforward method is employed by the software: all organs are uniformly scaled using the same factor, rather than an allometric exponent. This linear approach could be improved considering the significant body weight difference between NHPs. Alternatively, it might be advantageous to extend the OSP suite by a chimpanzee individual, similar to the implementation of both dogs and beagles (Open Systems Pharmacology Suite Manual Version 7.4, 2018).

Overall, the data of all three species were successfully described, given the assumptions of

- identical CD11a expression in NHP and human
- identical internalization kinetics of free and drug-bound CD11a in NHP and human
- comparable binding kinetics of efalizumab to CD11a in NHP and human, no binding in rabbit
- different binding affinities of efalizumab to FcRn in rabbit, NHP and human.

These results suggest that, when translating the efalizumab model from rabbit to primates, FcRn affinity has to be increased and TMDD processes need to be added. This finding may hold true for other human IgGs that were shown to exhibit lower in-vitro affinity to rabbit FcRn compared to human FcRn (Szikora et al., 2017). However, for Fc containing therapeutics other than IgGs this might not be the case.

As the rabbit model for efalizumab cannot give any insight into TMDD related parameters it may not be very useful for a translation to human. Thus, we conclude that rabbit is less relevant as an animal model for compounds that are not cross-reactive. While this study focused on rabbits and NHPs as animal models for translation, rodents are also often used for PK assessments. It has been demonstrated that mouse and rat PK models can be translated to humans successfully (Wang et al., 2016). However, as in the case of efalizumab in rabbits, drugs are often not cross-reactive in rodents (Germovsek et al., 2021). Consequently, in such instances, determining target-binding parameters becomes challenging. NHPs on the other hand, are more relevant when extrapolating to human. The presented results suggest, that all TMDD related parameters (target-binding, internalization, and expression) can be translated from NHP to human and only the FcRn affinity should be decreased. However, this must be treated with caution as the available data from NHPs for efalizumab are limited to only one dose. In the human study, the wide range of administered doses gives a good picture of the different phases of TMDD (see Figure 5). The sensitivity of the NHP and human models to the values of $k_{int,C}$ and target-binding parameters is low for the doses shown in Figure 13, but increases for lower doses (see **Supplementary Tables**). Preclinical data from different doses in NHP would be valuable to confirm the model parameter values identified in this study. Target-binding kinetics are generally assumed to be comparable in NHPs and humans (Deng et al., 2012; Germovsek et al., 2021). Even though the in-vitro/in-vivo correlation

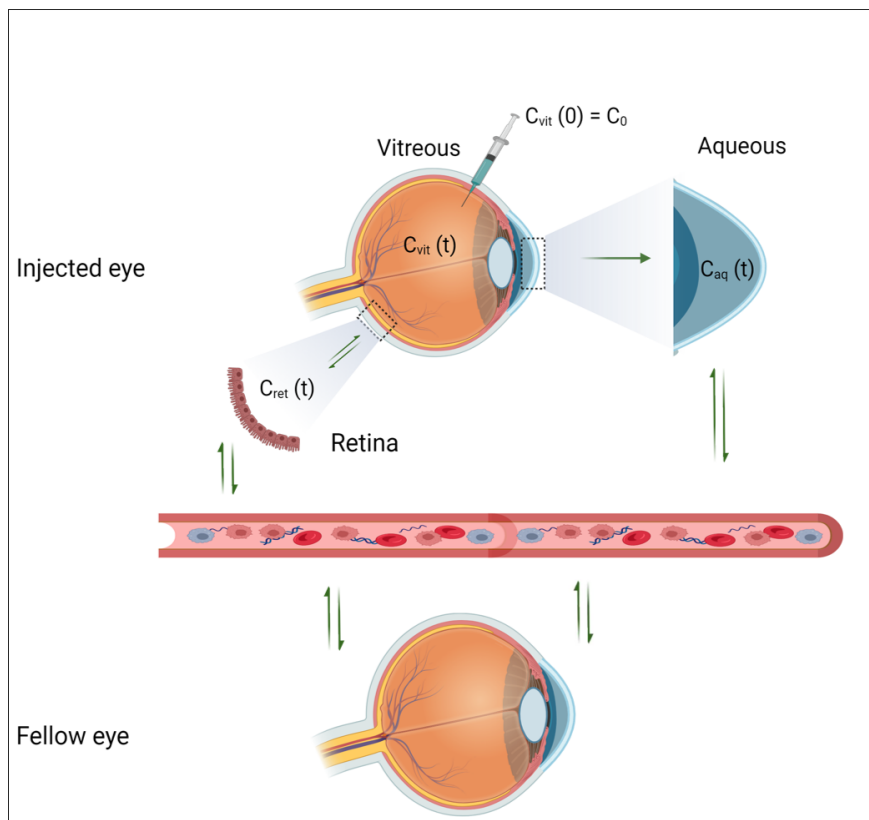
of binding parameters can differ substantially between species (Gabrielsson and Hjorth, 2023), the low sensitivity of the models to values of K_D and k_{off} suggests that a small deviation between species has no big impact on the model predictions, thus the assumption of similar binding rate constants between NHPs and humans seems to be of little consequence, even if it might not be valid. Unlike target-binding constants, target expression and turnover are more likely to differ between species (Deng et al., 2012; Gabrielsson and Hjorth, 2023). Our results suggest that CD11a reference concentration, $k_{int,T}$ and $k_{int,C}$ can be translated between NHP and human. However, parameters may have been not unambiguously identifiable for the NHP model because only one dose group was available. As shown in Figure 13, the monkey model is less sensitive to the respective parameters than the human model. When estimating TMDD related parameters (CD11a reference concentration, $k_{int,T}$, $k_{int,C}$, and k_{off} were estimated) using only the NHP data, the human data were not well described after transferring the NHP-derived parameters to the human model (results not shown). Thus, it could not be demonstrated that human PK is predictable by translation from NHP. However, the fact that a common parameter set achieved from a simultaneous fit of NHP and human data (6 dose groups in total) can describe both NHP and human data, suggests that this may be possible with a larger NHP data set used for parameter identification.

As conclusion from these findings, one could question the need for animal models in general, if there is not enough data collected to estimate relevant parameters in these preclinical studies, and if the most sensitive parameters cannot be translated between species. Sufficient human in-vitro data on key parameters such as target-binding kinetics and internalization rates might give more insightful information than limited animal data. While binding kinetics are commonly assessed in-vitro, assays for internalization rates and quantification of target expression are still rare, in spite of their high value for model predictions. With information on target expression and internalization rates, it might be possible to develop comprehensive human PBPK models without the need for animal testing.

Apart from the discussed limitations, the developed models are, to the best of our knowledge, the first PBPK models used to analyze efalizumab PK across three species. The use of a full (instead of a reduced) TMDD structure enables the

comparison of physiologically interpretable parameters between NHP and human. The assumptions of similar target-binding and identical target(-complex) internalization rates in NHP and human were confirmed for the available data. The presented models can be used to further investigate cross-species differences if additional data, preferably in NHPs, become available, or when other species of interest emerge. Finally, a growing understanding of mAb disposition across species can improve first in human predictions and enable more adequate dosing and sampling designs in early clinical trials.

Chapter 3: Injected eye, fellow eye and systemic pharmacokinetics of intravitreally administered bevacizumab



Figures of this chapter were partially created with BioRender.com.

Abstract

Simulation of retinal drug concentrations after intravitreal administration is still challenging, due to knowledge gaps in ocular physiology such as the molecule-dependent permeability of ocular segments and the mechanisms determining a drugs' diffusion through the vitreous. Current models consider the eye as an isolated organ, however, especially if we are striving for predictions that rely on plasma data only, it is crucial to understand the blood-ocular barrier to draw conclusions for drug exposure in the eye. Aims of this study were 1) to examine large molecule disposition within the rabbit injected eye as well as in the systemic circulation and the uninjected fellow eye using bevacizumab as a tool compound, 2) to show gaps in the current understanding of physiological processes governing drug distribution in the eye and 3) to identify model parameters relevant for eye-plasma drug exchange.

We developed a mechanistic ocular compartmental model to describe large molecule disposition in injected eye, plasma and fellow eye, following unilateral intravitreal bevacizumab administration in rabbit, based on literature data. Observations in the fellow eye add additional value to the developed model as they provide valuable new information about the blood-ocular barrier. Starting with a base model using physiological and correlation-derived parameters, we refined the structural model and parameter estimates (e.g., by adjusting the retinal volume) to better describe the observed data in the fellow eye.

The simulated concentration–time profiles from our refined model adequately described observed concentrations in the injected eye, plasma and fellow eye. Compared to base model predictions, retinal concentrations were better described when the retinal volume fraction accessible for bevacizumab was estimated, with bevacizumab entering approx. 41% of the retinal tissue volume. The estimated retinal pigment epithelium (RPE) permeability was $5.90 \cdot 10^{-9} \text{ cm} \cdot \text{s}^{-1}$, which is in close agreement with the values obtained from in-vitro experiments. The introduction of a backflow from the plasma compartment to the aqueous chamber via the ciliary body ($0.02 \text{ ml} \cdot \text{day}^{-1}$) yielded improved predicted aqueous concentrations in the fellow eye.

The presented study furthers our understanding of the fundamentals underlying ocular as well as systemic pharmacokinetics (PK). It introduces an advanced model structure (e.g., physiologically plausible retinal volume and permeability) based on data that was not analyzed previously for ocular model development. By refining the parameters of retinal volume accessible to large molecules and retinal permeability, we gained insights into the compound-specific drug distribution in the posterior eye, thereby enhancing our ability to predict drug exposure in the retina. Additionally, the increased understanding of the blood-ocular barrier, the physiological barrier separating exterior eye tissues from adjacent blood vessels, enables more accurate predictions based on plasma sampling only. In summary, the developed model increases our knowledge of ocular drug PK within a systemic context, with a more physiological representation of the retinal compartment and new insights into the processes involved at the blood-ocular barrier.

Introduction

Intravitreal (ivt) drug administration is a widely used technique for the treatment of retinal diseases (Peyman et al., 2009). It allows large molecule drugs to reach the tissues in the back of the eye, significantly increasing target site exposure while minimizing systemic exposure and associated adverse events. In recent years, therapeutic antibodies have become the leading category of newly developed drugs, also finding application in ocular therapy. The current standard treatment of age-related macular degeneration (AMD) involves the use of antibodies (or their fragments) directed against vascular endothelial growth factor (VEGF) (Amo et al., 2017). One particularly well studied example of these therapeutic antibodies is bevacizumab, an IgG monoclonal antibody originally developed for oncology therapy but used off-label in ocular therapy (Grisanti and Ziemssen, 2007).

Intravitreally administered large molecules are eliminated from the vitreous via two routes: (1) the anterior route where they diffuse to the aqueous chamber and are then further transported to the systemic circulation with the aqueous humor outflow, and (2) the posterior route where they diffuse to and then through the retina. Antibodies are

predominantly cleared through the anterior route, where the rate of elimination is determined by the molecules' diffusion rate in the vitreous humor (Urtti, 2006; Hutton-Smith et al., 2016). To enter the retina, molecules need to permeate through a collagen membrane called the inner limiting membrane (ILM). From the retina, they pass a cell monolayer called the retinal pigment epithelium (RPE), to reach the highly fenestrated choroidal blood vessels (Bill et al., 1980) and the systemic circulation. The retina is also perfused with blood vessels; however, large molecules are not expected to permeate capillary walls (Amo et al., 2017). Even though the anterior route is more relevant to determine the ocular half-life of a large molecule, it is the permeability of the posterior eye segments that will determine how much of the ivt administered dose reaches the target site in the retina. Thus, both processes are highly relevant for PK predictions. Mechanistic PK models can use physiological information of both elimination routes to predict drug half-life and exposure at the target site. Several ocular PK models have been developed so far: Schmitt et al. (Schmitt et al., 2017) used physics-based equations to predict ocular PK using a compound's molecular weight. Rimpelä et al. (Rimpelä et al., 2018) developed a mechanistic model for small molecules, considering the diffusion rate from vitreous to aqueous and the permeability across posterior tissues. Both groups validated their models based on vitreal half-life values. Hutton-Smith et al. (Hutton-Smith et al., 2017, 2018) developed a semi-mechanistic 3-compartment model for large molecules. They described concentration-time profiles of several different compounds in the rabbit retina, vitreous and aqueous (Hutton-Smith et al., 2017). Bussing, Shah and Naware (Bussing and Shah, 2020; Naware et al., 2023) recently published ocular PBPK models for large molecules in rabbit, monkey and human, applying the values for posterior eye permeabilities established for rabbits by Hutton-Smith et al. (Hutton-Smith et al., 2017).

Informing mechanistic ocular models is challenging due to uncertainties in several physiological parameters: Firstly, the elimination rate from vitreous to aqueous is difficult to determine. It is thought to depend on diffusion dynamics in the vitreous humor and thus, on molecular weight (Naware et al., 2023). There seems to be a linear relationship between vitreal diffusion time and vitreal half-life, however, this relationship is not uniform across species (Caruso et al., 2020). Further uncertainties arise from the inner limiting membrane permeability (pILM) and the retinal pigment

epithelium permeability (pRPE). Hutton-Smith et al. (Hutton-Smith et al., 2017) compared their estimated pILM and pRPE values to the respective molecular weights of the compounds, but the relationship they found was not linear. Literature suggests that posterior segment permeability may depend not only on the molecular weight, but also on the charge of a molecule (Peynshaert et al., 2018). However, the RPE permeabilities estimated by Hutton-Smith et al. deviate significantly from experimental measurements (Terasaki et al., 2015; Hutton-Smith et al., 2017; Ramsay et al., 2019). Finally, the effective retinal volume for protein therapeutics is difficult to determine, as the entry of large molecules into cells is typically limited. The reported retinal volume usually considers the whole retinal tissue, while only the interstitial space is accessible to large molecules. Therefore, an accurate description of the drug concentrations within the retina is difficult. Most current models consider the eye as an isolated organ, neglecting the systemic circulation as well as the fellow eye. This limitation becomes particularly relevant in human studies, where sampling from ocular tissues is rare and usually restricted to the aqueous humor, whereas plasma samples are readily available. In addition, systemic toxicological effects observed after ivt administration of monoclonal antibodies demonstrate the importance of understanding ocular drug PK within a systemic context.

The aim of this study was therefore, to describe the disposition of bevacizumab within the eye as well as the systemic circulation following ivt administration in rabbit, to assess current literature assumptions, and to further understand ocular PK in rabbit in general. While published data on bevacizumab following ivt administration in rabbits is limited to vitreous, aqueous, and serum samples, we utilized new in-house preclinical data for model development, adding retinal measurements from the injected eye to improve our ocular PK models. We developed a mechanistic model, describing the injected eye, systemic exposure and fellow eye PK of bevacizumab in rabbit. To the best of our knowledge, the current study is the first to introduce an ocular model structure including both, injected and fellow eye. The additional observations in the fellow eye provide valuable information to inform our structural model and to increase the confidence in parameter estimates. We further aimed to improve the understanding of the blood-ocular barrier to enable predictions based on plasma data, and thus, to contribute to the development of safer and more effective treatments for ocular diseases.

Results

We here developed a mechanistic ocular model to describe the drug concentrations in injected eye, fellow eye and plasma, for bevacizumab in rabbits. The model parameters were estimated from three different data sets (Table 2): Bakri et al. (Bakri et al., 2007) measured bevacizumab concentrations in vitreous, aqueous (injected and fellow eye) and plasma of 20 Dutch Belted rabbits after unilateral administration. Sinapis et al. (Sinapis et al., 2011) measured bevacizumab concentrations in vitreous, aqueous (injected and fellow eye) and plasma of 20 New Zealand White rabbits after unilateral administration. Literature data sets were complemented by unpublished data of eight New Zealand White rabbits receiving bilateral administration of bevacizumab (see **Chapter 6: Material and Methods**). In this in-house study, measurements were obtained from vitreous, aqueous, plasma, and also from retina of both injected eyes. All three studies took samples of 4 eyes per time point.

Table 2. PK data of ivt administered bevacizumab used for model development.

Source	Dose [mg/eye]	Injection	Strain	Number of animals	Compartments with observations
Bakri et al., 2007	1.25	unilateral	Dutch Belted	20	Vitreous, aqueous (injected + fellow eye); plasma
Sinapis et al., 2011	0.43*	unilateral	New Zealand White	20	Vitreous, aqueous (injected + fellow eye); plasma

In-house data**	1.25	bilateral	New Zealand White	8	Vitreous, aqueous, retina (injected eye); plasma
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*adjusted to match observations in the injected eye, **previously unpublished

The reported dose was 1.25 mg/eye for each study. However, for the study of Sinapis et al. (Sinapis et al., 2011), the dose was adjusted to capture the injected vitreous concentration-time profile. An unintentional reduction in the intended dose might occur due to the drug oozing out of the eye as a result of intraocular pressure. This observation has been reported previously in other publications (Amo and Urtti, 2015; Bussing and Shah, 2020).

Model development

A mechanistic ocular PK model was built in R, Version 4.2.2 (using the rxode2 package, Version 3.0.2). Phoenix WinNonlin™ (Version 8.2.2, Certara, USA) was used for parameter estimation. The model code can be found in the Appendix (see **Supplementary Material Chapter 3**). Our base model structure described the injected and fellow eye using the three-compartment structure published by Hutton-Smith et al. (Hutton-Smith et al., 2017) along with two-compartment systemic disposition. The plasma and peripheral compartment, as well as the fellow eye are substantial extensions of the Hutton-Smith et al. 2017 model. Starting with parameters available from literature, we refined the model. The refined model was developed considering physiological adjustments (retinal volume, bidirectional exchange between aqueous and plasma compartments) and re-estimating relevant parameters. Goodness of fit was evaluated by simulating the concentration-time profiles for the vitreous, aqueous, retina and plasma compartments and comparing them against experimentally observed concentrations with subsequent quantification of the model performance using the absolute average fold error (AAFE) (see **Materials and Methods for chapter 3**).

The developed model structure is represented by eight compartments: injected eye vitreous, aqueous and retina; fellow eye vitreous, aqueous and retina; plasma compartment and peripheral compartment (see Figure 14).

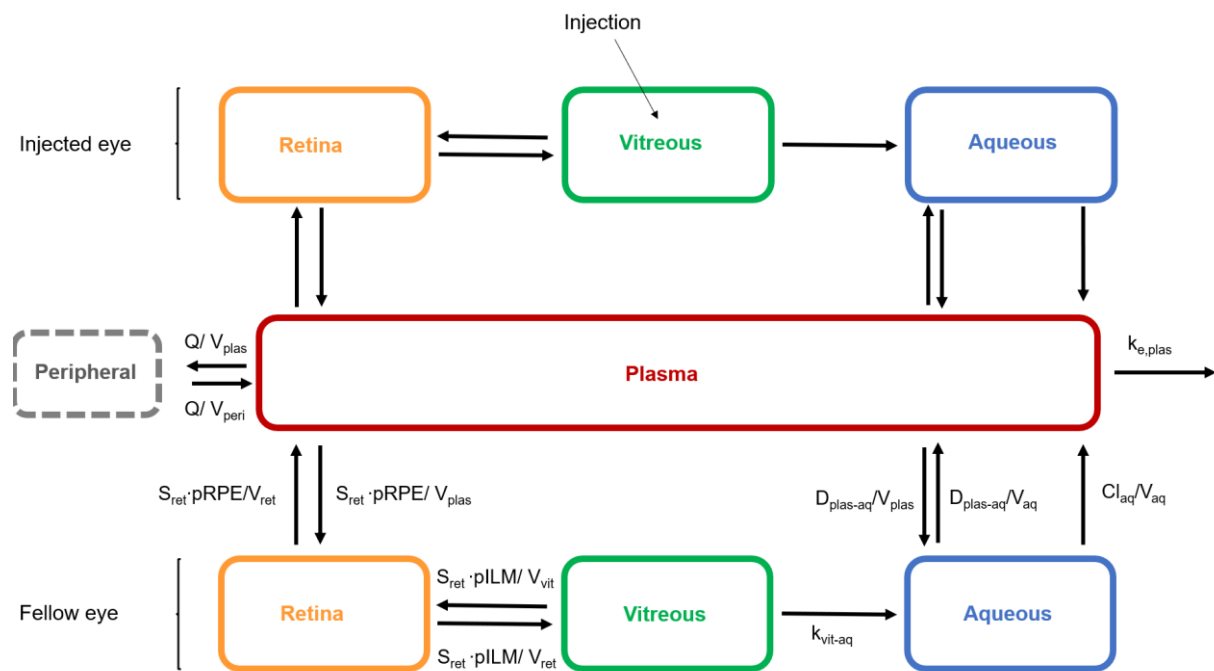


Figure 14. Modeled ocular and systemic PK processes. Following ivt administration to the vitreous of the injected eye, drug diffuses from the center of the vitreous into aqueous and retina, and subsequently into plasma from where it reaches the fellow eye, distributes into the peripheral compartment or gets eliminated. Transport pathways are indicated by arrows. For simplification, the respective transfer rate constants are only indicated in the fellow eye. Cl_{aq} : Aqueous humor clearance via the Schlemm's canal; $D_{plas-aq}$: transfer rate between plasma and aqueous chamber (only in refined model); $k_{e,plas}$: plasma elimination rate constant; k_{vit-aq} : diffusion rate constant from vitreous to aqueous chamber; $pILM$: permeability of the inner limiting membrane; $pRPE$: permeability of the retinal pigment epithelium; Q : distribution rate between plasma and peripheral compartment; S_{ret} : retinal surface area; V_{aq} : volume of aqueous; V_{peri} : volume of peripheral compartment; V_{plas} : volume of plasma; V_{ret} : volume of retina; V_{vit} : volume of vitreous.

The model incorporates the following assumptions: At time point $t = 0$ days, bevacizumab is administered into the center of the vitreous with volume V_{vit} , resulting in an initial concentration of $dose/V_{vit}$ in the injected vitreous. Drug may leave the vitreous by the anterior pathway, with the first-order rate constant k_{vit-aq} describing the diffusion into the aqueous chamber. This diffusion was assumed to be unidirectional, due to the high flow rate of the aqueous humor out of the aqueous chamber via the Schlemm's canal (Missel, 2012). From the aqueous chamber, drug reaches the systemic circulation via the Schlemm's canal at a rate corresponding to the aqueous humor outflow (Cl_{aq}). For the same reasons as for k_{vit-aq} , Cl_{aq} was assumed to be unidirectional. Drug may also leave the vitreous by the posterior route. It can circulate between the vitreous and the retina, by permeating the ILM. From the retina, drug passively diffuses through the RPE and enters the systemic circulation via highly fenestrated choroidal blood vessels (Amo et al., 2017). The drug can also re-enter the retina from the systemic circulation. Vitreal, aqueous and retinal volumes were assumed to be the same in both eyes. From the plasma compartment, drug distribution into the peripheral compartment (representing all peripheral tissues accessible to bevacizumab) is governed by the flow rate Q , and irreversible drug clearance is governed by elimination rate constant $k_{e,plas}$. This constant reflects the degradation of the drug by lysosomes, as well as binding of the drug to its target followed by drug-target-complex internalization and degradation. Target binding was assumed to be negligible in the eye compartments, due to the low concentrations of VEGF in the eye (Wang et al., 2016).

The described processes were assumed to be first order and are depicted by arrows with flow rate constants in Figure 14. They were formulated as a system of ordinary differential equations, reflecting the time course of bevacizumab concentrations in each compartment, assuming initial conditions $c_{vit}(0) = dose/V_{vit}$ and $c_{aq}(0) = c_{ret}(0) = c_{fvit}(0) = c_{faq}(0) = c_{fret}(0) = c_{plas}(0) = c_{peri}(0) = 0$:

Injected eye:

$$\frac{dc_{vit}}{dt} = -k_{vit-aq} \cdot c_{vit} + \frac{S_{ret}}{V_{vit}} \cdot p_{ILM} \cdot c_{ret}/f_{ret} - \frac{S_{ret}}{V_{vit}} \cdot p_{ILM} \cdot c_{vit}$$

$$\frac{dc_{aq}}{dt} = \frac{V_{vit}}{V_{aq}} \cdot k_{vit-aq} \cdot c_{vit} - \frac{Cl_{aq}}{V_{aq}} \cdot c_{aq} + \frac{D_{plas-aq}}{V_{aq}} \cdot c_{plas} - \frac{D_{plas-aq}}{V_{aq}} \cdot c_{aq}$$

$$\begin{aligned} \frac{dc_{ret}}{dt} = & \frac{S_{ret}}{V_{ret}} \cdot pILM \cdot c_{vit} - \frac{S_{ret}}{V_{ret}} \cdot pILM \cdot c_{ret}/fret - \frac{S_{ret}}{V_{ret}} \cdot pRPE \cdot c_{ret}/fret \\ & + \frac{S_{ret}}{V_{ret}} \cdot pRPE \cdot c_{plas} \end{aligned}$$

Fellow eye:

$$\frac{dc_{fvit}}{dt} = -k_{vit-aq} \cdot c_{fvit} + \frac{S_{ret}}{V_{vit}} \cdot pILM \cdot c_{fret}/fret - \frac{S_{ret}}{V_{vit}} \cdot pILM \cdot c_{fvit}$$

$$\frac{dc_{faq}}{dt} = \frac{V_{vit}}{V_{aq}} \cdot k_{vit-aq} \cdot c_{fvit} - \frac{Cl_{aq}}{V_{aq}} \cdot c_{faq} + \frac{D_{plas-aq}}{V_{aq}} \cdot c_{plas} - \frac{D_{plas-aq}}{V_{aq}} \cdot c_{faq}$$

$$\begin{aligned} \frac{dc_{fret}}{dt} = & \frac{S_{ret}}{V_{ret}} \cdot pILM \cdot c_{fvit} - \frac{S_{ret}}{V_{ret}} \cdot pILM \cdot c_{fret}/fret - \frac{S_{ret}}{V_{ret}} \cdot pRPE \cdot c_{fret}/fret \\ & + \frac{S_{ret}}{V_{ret}} \cdot pRPE \cdot c_{plas} \end{aligned}$$

Plasma:

$$\begin{aligned} \frac{dc_{plas}}{dt} = & \frac{Cl_{aq}}{V_{plas}} \cdot c_{aq} + \frac{Cl_{aq}}{V_{plas}} \cdot c_{faq} + \frac{S_{ret}}{V_{plas}} \cdot pRPE \cdot c_{ret}/f_{ret} + \frac{S_{ret}}{V_{plas}} \cdot pRPE \\ & \cdot c_{fret}/f_{ret} - 2 \cdot \frac{S_{ret}}{V_{plas}} \cdot pRPE \cdot c_{plas} + \frac{D_{plas-aq}}{V_{plas}} \cdot c_{aq} \\ & + \frac{D_{plas-aq}}{V_{plas}} \cdot c_{faq} - 2 \cdot \frac{D_{plas-aq}}{V_{plas}} \cdot c_{plas} - \frac{Q}{V_{plas}} \cdot c_{plas} + \frac{Q}{V_{plas}} \cdot c_{peri} \\ & - k_{e,plas} \cdot c_{plas} \end{aligned}$$

Peripheral compartment:

$$\frac{dc_{peri}}{dt} = \frac{Q}{V_{peri}} \cdot c_{plas} - \frac{Q}{V_{peri}} \cdot c_{peri}$$

with c_{aq} : bevacizumab concentration in the injected eye aqueous; c_{faq} : bevacizumab concentration in the fellow eye aqueous; c_{fret} : bevacizumab concentration in the fellow eye retina; c_{fvit} : bevacizumab concentration in the fellow eye vitreous; c_{peri} : bevacizumab concentration in the peripheral compartment; c_{plas} : bevacizumab concentration in plasma; c_{ret} : bevacizumab concentration in the injected eye retina; c_{vit} : bevacizumab concentration in injected eye vitreous; Cl_{aq} : aqueous humor clearance; $D_{plas-aq}$: transfer rate between plasma and aqueous chamber (only in refined model); f_{ret} : retinal volume fraction accessible to drug; $k_{e,plas}$: plasma elimination rate constant; k_{vit-aq} : diffusion rate constant from vitreous to aqueous chamber; $pILM$: permeability of the inner limiting membrane; $pRPE$: permeability of the retinal pigment epithelium; Q : distribution rate between plasma and peripheral compartment; S_{ret} : retinal surface area; V_{aq} : volume of aqueous; V_{peri} : volume of peripheral compartment; V_{plas} : volume of plasma; V_{ret} : volume of retina; V_{vit} : volume of vitreous.

The equations were formulated in terms of drug concentrations, rather than drug amounts, leading to volume factors in all transport rates to account for compartment size. The equations represent the refined model structure. In the base model structure, f_{ret} corresponded to 1 and $D_{plas-aq}$ to 0. c_{ret} refers to the bevacizumab concentrations in the volume of the retina that is accessible to the drug. The observed retinal data still

reflect the concentrations related to the whole retinal volume, as measurements were taken from the complete retina sample including the inaccessible fraction. To account for this, the simulations were multiplied by f_{ret} and the observed data were presented as concentrations with respect to the total retinal volume (as usually reported). During model development, parameters for ocular and plasma volumes, as well as retinal surface area were fixed to literature values (Missel, 2012; Gadkar et al., 2015). Cl_{aq} was fixed to the physiological aqueous humor production rate from literature (Missel, 2012). For the base model, $pILM$ and $pRPE$ were fixed to the estimations obtained by Hutton-Smith et al. (Hutton-Smith et al., 2017) for an IgG antibody. The remaining parameters (k_{vit-aq} , $k_{e,plas}$, V_{peri} , Q , dose for the Sinapis et al. data set) were estimated. For the refined model $k_{e,plas}$, V_{peri} , Q and the dose for the Sinapis et al. data set were fixed from the base model and k_{vit-aq} , $pILM$, $pRPE$, f_{ret} and $D_{plas-aq}$ were estimated. V_{peri} and $k_{e,plas}$ were allowed to differ for the Sinapis et al. data set, as the lower exposure in plasma and peripheral compartment following a lower dose was expected to lead to non-linearity in plasma and peripheral tissues due to non-saturated target binding (Wang et al., 2016). Table 3 summarizes the estimated and fixed parameters of the developed base and refined models.

Table 3. Parameters of the bevacizumab ocular PK base and refined models.

Parameter	Parameter value (CI95*)	Source	Description
V_{vit} [ml]	1.52	Missel et al., 2012	Volume of vitreous
V_{aq} [ml]	0.325	Missel et al., 2012	Volume of aqueous chamber

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V_{ret} [ml]	0.044	Missel et al., 2012	Volume of retina
S_{ret} [cm ²]	4.35	Hutton-Smith et al., 2017	Retinal surface area
Cl_{aq} [ml • day ⁻¹]	4.32	Missel et al., 2012	Aqueous humor clearance
V_{plas} [ml]	80	Gadkar et al., 2015	Volume of plasma compartment
V_{peri} [ml]	104 (65.3, 142) 402 (164, 640)	Estimated for Bakri et al., 2007 and in-house data set Estimated for Sinapis et al., 2011 data set	Volume of peripheral compartment
Q [ml • day ⁻¹]	1030 (-127, 2180)	Estimated	Distribution rate between plasma and peripheral compartment
$k_{\text{e,plas}}$ [day ⁻¹]	0.339 (0.293, 0.384) 1.15 (0.718, 1.58)	Estimated for Bakri et al., 2007 and in-house data set Estimated for	Plasma elimination rate constant

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			Sinapis et al., 2011 data set	
	Base model	Refined model		
k_{vit-aq} [day ⁻¹]	0.137 (0.136- 0.144)	0.147 (0.140- 0.153)	Estimated	Diffusion rate constant from vitreous to aqueous chamber
p_{ILM} [cm • s ⁻¹]	$1.70 \cdot 10^{-7}$	$4.16 \cdot 10^{-4}$ (- 2.84, 2.84)	Base model: Hutton-Smith et al., 2017 (estimation for an IgG) Refined model: estimated	Permeability of the inner limiting membrane (ILM)
p_{RPE} [cm • s ⁻¹]	$1.84 \cdot 10^{-7}$	$5.90 \cdot 10^{-9}$ ($1.69 \cdot 10^{-9}$, $10.1 \cdot 10^{-9}$)	Base model: Hutton-Smith et al., 2017 (estimation for an IgG) Refined model: estimated	Permeability of the retinal pigment epithelium (RPE)
f_{ret}	-	0.41 (0.36, 0.46)	Estimated	Retinal volume fraction accessible to drug

$D_{\text{plas-aq}}$ [ml • day ⁻¹]	-	0.024 (0.014, 0.035)	Estimated	Transfer rate between plasma and aqueous chamber
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*CI95: 95% confidence interval

Base model

The base model included the three-compartment structure published by Hutton-Smith et al. (Hutton-Smith et al., 2017) plus a two-compartment systemic disposition. The observations in the injected eye and plasma could be described well using this model structure, however, bevacizumab concentrations in the vitreous of the fellow eye were overpredicted (Figure 15). This suggests that the flow from plasma to retina to vitreous was overestimated. The AAFE for the overall model was 2.1 and for the fellow eye 7.3.

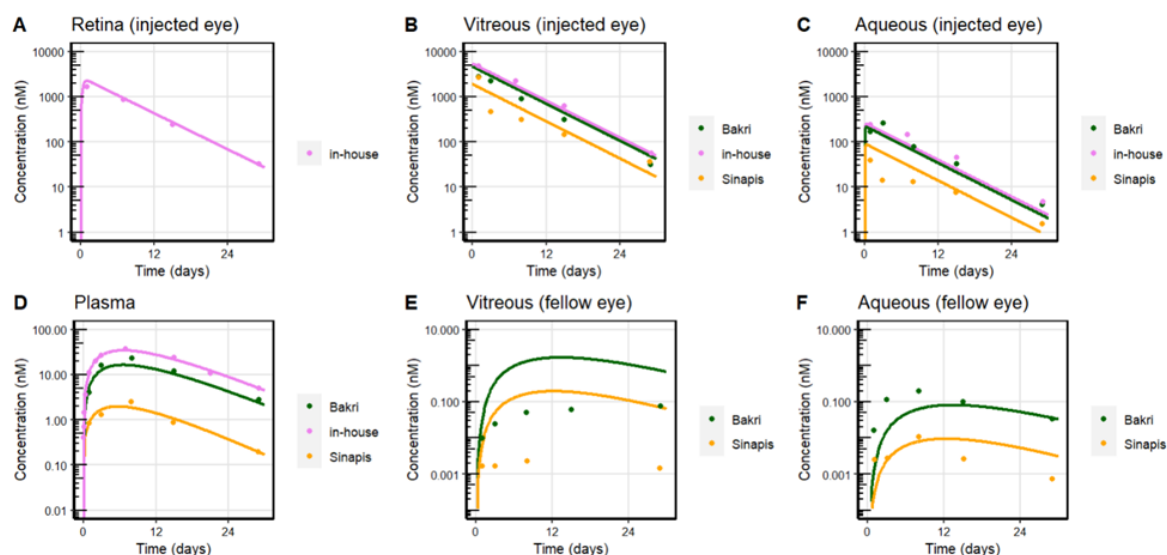


Figure 15. Base model simulations: free bevacizumab concentrations over time in rabbit (A) retina (injected eye), (B) vitreous (injected eye), (C) aqueous (injected eye), (D) plasma, (E) vitreous (fellow eye) and (F) aqueous (fellow eye). Simulations are

shown as lines and observed data are shown as points. Green: Bakri et al. (Bakri et al., 2007), orange: Sinapis et al. (Sinapis et al., 2011), violet: in-house data.

Refined model

The refined model was developed taking physiological adjustments of the retinal volume and the exchange between plasma and aqueous chamber into account. As large molecule drugs are likely not able to access the entire retinal tissue volume which consists in large part of cells, we reduced the retinal volume accessible to bevacizumab in the model structure. With this adjustment, $pRPE$, $pILM$ and k_{vit-aq} had to be re-estimated (Table 3). The retinal volume fraction accessible to bevacizumab was estimated at 0.41. According to this estimate, bevacizumab can enter only 41% of the retinal tissue volume. Experimental data suggest that isolated rabbit retina consists to 31% of interstitial fluid (Ames and Nesbett, 1966). For our model f_{ret} of 41% was necessary to adequately describe the injected eye retina concentrations. The re-estimated $pRPE$ was $5.90 \cdot 10^{-9} \text{ cm} \cdot \text{s}^{-1}$, which is around 30-fold lower than in the base model. The value of $pILM$ was estimated at $4.16 \cdot 10^{-4} \text{ cm} \cdot \text{s}^{-1}$, but as shown by the large 95% confidence interval, this parameter was not identifiable. In the injected eye retina compartment, $pILM$ determines the delay of the drug inflow from the vitreous. As the earliest observation in the retina is rather late (1 day), the model is not sensitive towards changes in this parameter. With these adjustments of f_{ret} and $pRPE$, the data in the fellow eye vitreous could be adequately described (Figure 16). To avoid underprediction in the fellow eye aqueous compartment, the model structure was further refined by adding an exchange pathway between the plasma compartment and the aqueous chambers of the injected and fellow eyes, with a transfer rate $D_{plas-aq}$ that was estimated from the data (Table 3). Figure 16 shows that the concentration-time profiles of injected eye, fellow eye and plasma were adequately described with the refined model structure. The refined model exhibits an improved overall AAFE of 1.5 and fellow eye AAFE of 2.0.

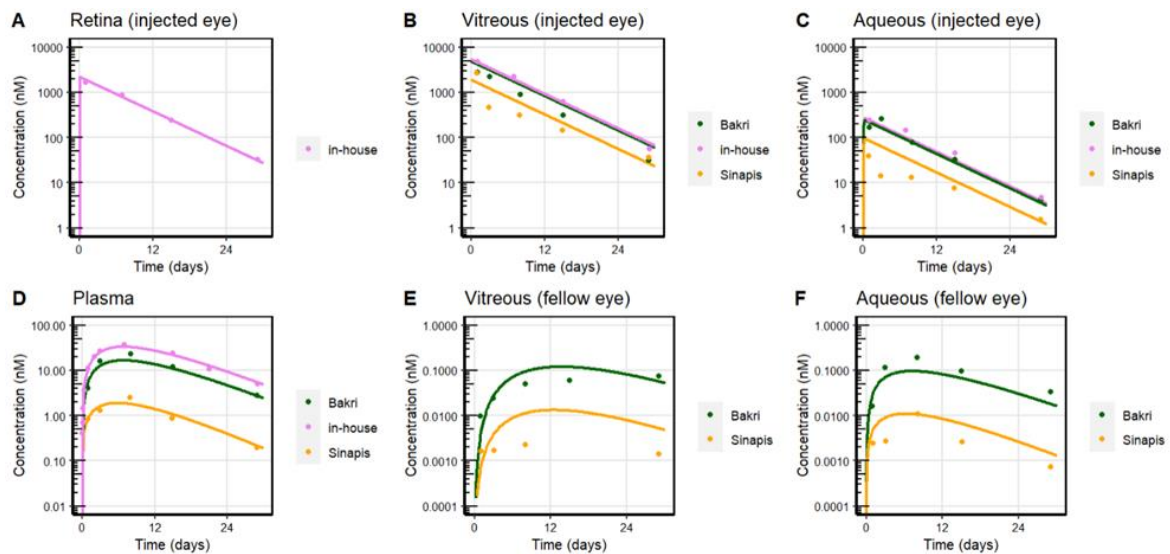


Figure 16. Refined model simulations with adjusted retinal volume, pRPE, and addition of $D_{\text{plas-aq}}$: free bevacizumab concentrations over time in rabbit (A) retina (injected eye), (B) vitreous (injected eye), (C) aqueous (injected eye), (D) plasma, (E) vitreous (fellow eye) and (F) aqueous (fellow eye). Note, that the retinal data represent the concentrations related to the whole retinal volume, as measurements reflect the concentrations in the complete retinal tissue including the inaccessible cellular fraction. Thus, simulations in the retina compartments were calculated accordingly. Simulations are shown as lines and observed data are shown as points. Green: Bakri et al. (Bakri et al., 2007), orange: Sinapis et al. (Sinapis et al., 2011), violet: in-house data.

Sensitivity analysis

Figure 17 displays the results of the local sensitivity analyses. Shown here are the results for injected eye retina and fellow eye vitreous compartments of the base and refined model. In the retina, the base model is more sensitive to the values of p_{ILM} and p_{RPE} . The adjustment of the accessible retinal volume in the refined model decreases the sensitivity of the simulations to both parameters. Instead, the refined model is moderately sensitive to f_{ret} , as the accessible fraction is an important parameter to describe the retinal concentrations. The fellow eye vitreous concentrations are highly sensitive to changes in p_{RPE} . Similar to the injected eye

retina, the fellow eye vitreous concentrations become insensitive to changes in pILM after model refinement.

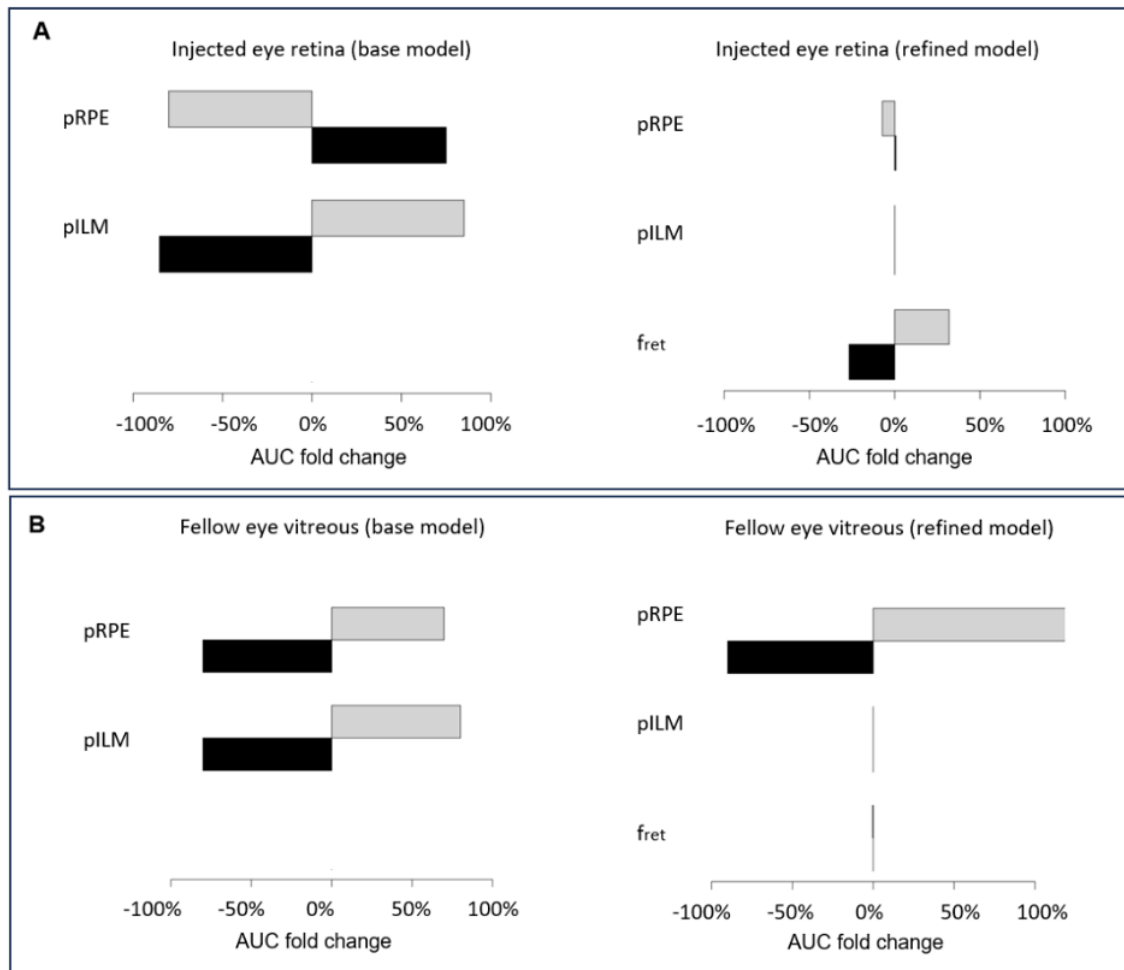


Figure 17. Local sensitivity analyses of (A) injected eye retina, and (B) fellow eye vitreous of the base and refined models. Shown are 10-fold variation of retinal pigment epithelium permeability (pRPE), 10-fold variation of inner limiting membrane permeability (pILM), and 2-fold variation of accessible fraction of retinal volume (f_{ret}). X-axes show the AUC fold change after parameter increase (grey) and parameter decrease (black).

Discussion

The model presented in this study offers a more comprehensive representation of ocular pharmacokinetics (PK) of large molecules compared to existing literature models featuring an extended structure that encompasses injected eye, fellow eye, plasma, and peripheral compartments. It provides a significant advancement incorporating observed data from fellow eye measurements, a practice that has not been previously implemented. This allows fellow eye predictions from systemic exposure, along with a more physiological interpretation of the blood-ocular barrier and the accessible retinal volume. To the best of our knowledge, this is the first mechanistic model to integrate fellow eye and plasma PK. By utilizing this more detailed structure, along with a robust data set which includes information from the fellow eye, this model improves the identifiability of unknown parameters to gain more confidence in their estimated values and establishes a connection between ocular PK and plasma exposure. Both, the base model and the refined model, can describe the injected eye concentrations in vitreous, aqueous and retinal compartments as well as the plasma profile of bevacizumab, however, only the refined model can describe the fellow eye concentrations adequately. The refined model gives a better representation of the actual physiological conditions, including the retinal volume accessible to large molecules along with more physiologically plausible values of the permeabilities around the retina (explained in detail in subchapter **Drug disposition in the posterior eye**). Compared to the base model, the refined model demonstrates improved model performance, decreasing the overall AAFE from 2.1 to 1.5.

Systemic drug disposition

The estimated plasma elimination rate constant of 0.339 day^{-1} is in very good agreement with the observations of Wang et al. (Wang et al., 2016b) who analyzed bevacizumab PK after intravenous administration in rabbits. They estimated the systemic clearance at $9.15 \pm 6.79 \text{ ml} \cdot \text{day}^{-1} \cdot \text{kg}^{-1}$ for a 2.3 kg rabbit which corresponds to an elimination rate constant of $0.263 \pm 0.195 \text{ day}^{-1}$, assuming a plasma volume of 80 ml. For the data set of Sinapis et al. (Sinapis et al., 2011), a higher plasma elimination rate constant was estimated (1.15 day^{-1}). Note that the model includes only one elimination rate as a simplification. In fact, bevacizumab clearance involves not

only direct endosomal degradation, but also saturable binding to VEGF and subsequent degradation of the drug-target-complex, resulting in higher elimination rates of lower doses as long as the target is not saturated. Thus, for the lower dose of Sinapis et al. (Sinapis et al., 2011), non-saturated target binding was expected, confirmed by higher estimated values of elimination rate (1.15 day^{-1}) and peripheral volume (402 ml). Alternative explanations for the lower plasma exposure observed in the Sinapis et al. data set (Sinapis et al., 2011) are possible, such as an even lower dose than we assumed here, but we cannot determine the exact cause with the data available. Nonetheless, accurately predicting plasma PK was essential for our study, as the description of the fellow eye data is important for the conclusions drawn from this analysis.

To predict ocular PK from plasma data, a good understanding of systemic disposition processes is necessary, including target-mediated drug disposition (TMDD). This requires knowledge about several parameters that are species specific, such as binding constants, target abundance, target turnover and drug-target-complex degradation rate. In-vitro measurements or estimates from an intravenous study could provide this knowledge, but most of the parameters mentioned are not available in the literature for the presented analysis. If ocular predictions from plasma data are required, such measurements would be most valuable. Only then could the necessary model parameters be “fixed”, and conclusions drawn for unknown ocular parameters k_{vit-aq} , $pILM$, $pRPE$ and $D_{plas-aq}$. This aspect is especially important for the translation of the model to humans, where samples are often obtained from plasma only.

Drug disposition in the posterior eye

Although both models describe the injected eye concentrations well, the fellow eye vitreous concentrations are overpredicted with the base model, which uses $pRPE$ as estimated by Hutton-Smith et al. (Hutton-Smith et al., 2017) (Figure 15). Therefore, we propose here an alternative model structure with a lower value for $pRPE$. To describe the roughly two-fold higher concentrations in the injected eye vitreous compared to retina, the base model uses the ratio of ILM and RPE permeabilities resulting in a slightly higher value for $pRPE$ than $pILM$. Due to the different compositions of the ILM and RPE it is unlikely that $pRPE$ and $pILM$ have similar

magnitudes. As the RPE is a tight cell monolayer, it is expected to pose a stronger barrier than the ILM, making a lower value for pRPE more physiologically plausible. Introducing a parameter to define the fraction of retinal tissue accessible to large molecules allowed us to reduce pRPE and still describe the concentration difference between the vitreous and the retina of the injected eye.

We estimated this retinal volume fraction accessible for bevacizumab at 0.41. According to this estimate, bevacizumab can enter only 41% of the retinal volume. It is physiologically plausible that a part of the retina is not accessible to bevacizumab, because as a large molecule it probably cannot enter cells. To get more confidence in this value, model development for additional compounds as well as further experimental data on the volume of retinal interstitial space would be beneficial. With the adjustment of the retinal volume accessible to bevacizumab, pRPE was re-estimated at $5.9 \cdot 10^{-9} \text{ cm} \cdot \text{s}^{-1}$, which is around 30-fold lower than the value estimated by Hutton-Smith et al. (Hutton-Smith et al., 2017) for a different IgG compound with similar size. Supporting our estimated value, reported experimental values are lower than the value suggested by Hutton-Smith et al. (Hutton-Smith et al., 2017). Ramsay et al. (Ramsay et al., 2019) measured a permeability of bevacizumab of $3.5 \cdot 10^{-8} \text{ cm} \cdot \text{s}^{-1}$ in isolated bovine RPE-choroid. Terasaki et al. (Terasaki et al., 2015) measured the permeability of bevacizumab through highly polarized porcine RPE cells. Based on permeated bevacizumab concentrations, they found a pRPE of $1.0 \cdot 10^{-8} \text{ cm} \cdot \text{s}^{-1}$ (calculated from the 6 to 12 h time point of the published figure), which is only 1.7-fold higher than our estimated value. Therefore, it is more likely that the difference in injected eye vitreous and retina concentrations is explained by a limited accessible volume of the retina rather than the previously described difference in pILM and pRPE, with pILM being lower than pRPE. Additionally, the value estimated for pILM by Hutton-Smith et al. ($1.7 \cdot 10^{-7} \text{ cm} \cdot \text{s}^{-1}$) seems low for a molecule of 150 kDa. For example, the pore size of the human ILM is suggested to be approximately 10 nm and fluorescein (FITC)-dextran of 500 kDa are reported to penetrate the ILM freely *ex vivo* (Amo et al., 2017). Further experimental data of multiple compounds would be highly beneficial to inform pILM for future modeling.

To further analyse the differences between the base and refined model structures, we performed sensitivity analyses of the injected eye retina concentrations (Figure 17).

As mentioned, the base model describes the observed injected eye vitreous-retina concentration difference solely by the ratio of pILM and pRPE, assuming a uniform distribution of drug in the entire retinal tissue volume. Therefore, the retinal AUC shows high sensitivity to changes in pRPE and pILM. As the base model values for pRPE differ from values determined in-vitro, and pILM is difficult to measure experimentally, it is challenging to predict retinal concentration accurately with this model. The refined model, on the other hand, describes the vitreous-retina concentration difference using the distribution volume factor f_{ret} , resulting in a lower estimated value for pRPE and retinal concentrations in the distribution volume (interstitial space) that are essentially equal to vitreous concentrations after a short delay determined by pILM. As a result, the injected eye retina concentrations are no longer sensitive towards changes in pRPE and pILM in the tested 10-fold range assessed in the sensitivity analyses. Additionally, we performed the sensitivity analyses on the fellow eye vitreous concentrations to show the importance of the fellow eye data to inform the estimate for pRPE. As the injected eye retina concentrations are not sensitive towards changes in pRPE, it is important to note that the fellow eye vitreous concentrations are, allowing us to determine a value for pRPE. Given that many therapeutic targets are located in the retina, accurately estimating pRPE, and as a result the drug concentrations in the interstitial space of the retina, is essential to predict drug exposure at the target site. Despite the detailed model structure and physiologically reasonable parameter estimates, the presented model has some limitations. Even though the model structure is more complex than other ocular models presented in literature, the structure might still be too simple to reflect all relevant ocular PK processes. We assumed that pRPE is the same in both directions, from retina to plasma and vice-versa. However, it is possible that the inward- and outward pRPEs differ. Possible reasons could be active transport processes or FcRn shuttling of bevacizumab. Furthermore, the diffusion path through the fellow eye vitreous probably differs from that of the injected eye. In the injected eye, drug diffusion starts from the center of the injection. In contrast, in the fellow eye the drug enters the vitreous from the entire posterior surface covered by the retina. Such spatial effects cannot be described with the current ODE-based model. Additionally, intraocular pressure differences might influence the vitreous diffusion dynamics. However, a higher pressure after ivt administration has only been observed for a short time frame (below 20 minutes) after injection (Murphy et al., 2023) and

might not be relevant for the observations used for model development. If diffusion dynamics differ in the fellow eye compared to the injected eye, the estimated k_{vit-aq} might differ between eyes, or a different structural model might be necessary to capture the fellow eye diffusion dynamics. Another limitation of the model arises from the uncertainty as to whether target-mediated drug disposition occurs in the retina. The assumption of negligible target binding in the eye was made based on the low VEGF measurements in the vitreous and aqueous humor of rabbits (Zhao et al., 2010). However, there were no data available of VEGF concentrations in rabbit retina. If there is target binding in the retina, this would especially be relevant at low drug concentrations (as in the fellow eye) and would also influence the amount of drug that enters the fellow eye vitreous from the fellow eye retina. This could be an alternative explanation for the low observed fellow eye vitreous concentrations, according to which the base model was adjusted. Observed data in the retina of the fellow eye would be very helpful to test the described hypotheses. Since samples are rarely taken from the retina, it is challenging to determine the values of pILM and pRPE. These parameters have so far only been estimated for rabbits by Hutton-Smith et al. (Hutton-Smith et al., 2017) for a set of large molecules, and these estimates have been adopted by Naware et al. (Naware et al., 2023). However, our model proposes an alternative estimate for pRPE, which aligns more closely with our physiological understanding of the eye and in-vitro observations. Thus, although both the base model and the refined model can fit the observations in the injected eye, the adjusted structure is more physiologically plausible and confirmed by the fellow eye data.

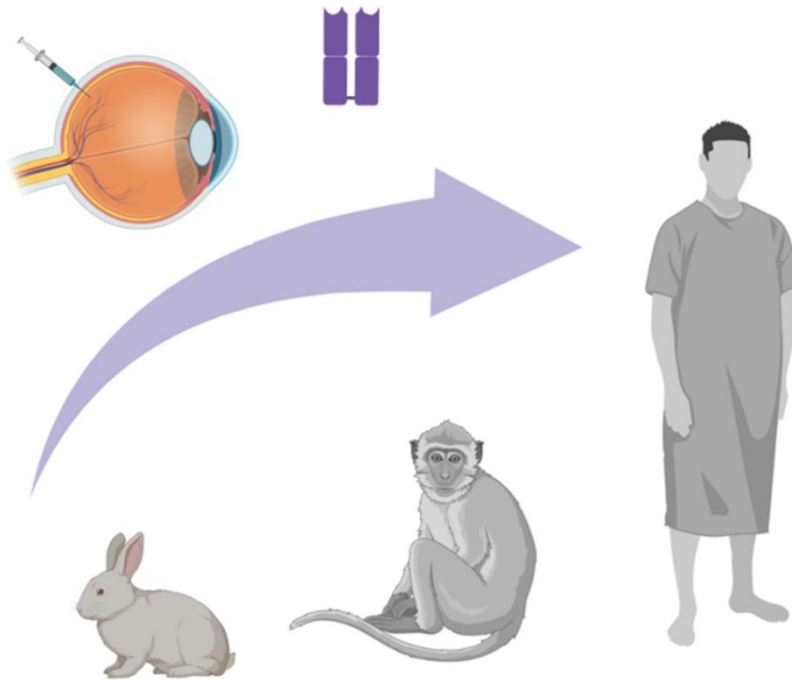
Backflow from plasma into aqueous

In the base model structure, the only input into the aqueous compartment arises from the vitreous. Given the lower concentrations in the fellow eye vitreous compared to the fellow eye aqueous, the data cannot be described using the base model structure. Thus, the model was refined adding a transfer pathway from the plasma compartment into the aqueous chamber and vice-versa. Due to the high flow rate of aqueous humor out of the aqueous chamber, it is unlikely that this input is caused by a backflow of the drug via the Schlemm's canal. An alternative explanation for higher drug concentrations in the aqueous might be that systemic bevacizumab enters the ciliary

body and from there, passes the epithelial cells to reach the aqueous chamber. Blood vessels in the rabbit ciliary body were shown to be permeable for large molecules (Bill, 1968; Smith and Rudt, 1975; Bill et al., 1980). The addition of this process may seem of low significance for ocular therapy, as it does not affect the PK in the injected eye retina and vitreous. However, it affects the aqueous PK at later time points, and thus is relevant to make more precise predictions and draw conclusions if only aqueous humor samples are available, as is often the case for human studies. In addition, $D_{\text{plas-aq}}$ is relevant to determine ocular toxicity after systemic exposure. According to Bakri et al. (Bakri et al., 2007), the observations in the fellow eye with lower vitreous than aqueous concentrations suggest that due to this concentration gradient there will be a backflow from the aqueous into the vitreous. We did not assume this backflow in our model, as the vitreous concentrations of the fellow eye were already overestimated having the exclusive influx from the retina. Thus, if an additional pathway from aqueous into vitreous would be included, the pRPE would have to be lowered even further and move away from the experimental values. This does not mean that the fellow eye vitreous concentrations cannot arise from both sources, the posterior and anterior eye. Again, measurements in the fellow eye retina would be helpful to investigate this further.

To conclude, this work contributes to a better understanding of the structural basis underlying ocular PK. It presents a refined model structure based on data that were not analyzed before for earlier ocular model developments. The refined estimations of pILM and pRPE help to investigate the compound-specific drug disposition in the posterior eye, improving the prediction of retinal drug exposure. Furthermore, increased understanding of the blood-ocular barrier will help us on our way to enable future predictions based solely on plasma data. Overall, the developed model increases our knowledge of ocular drug PK.

Chapter 4: Cross-species ocular pharmacokinetic models of ranibizumab



Figures of this chapter were partially created with BioRender.com.

Abstract

Cross-species translation plays a crucial role in ocular pharmacokinetic (PK) studies due to the limitations of sampling from posterior eye tissues in humans. By gaining a deeper understanding of inter-species differences, we can effectively support the development of ocular drugs. In this study, we examined large molecule ocular PK across rabbits, monkeys, and humans.

PK data of rabbit, monkey and human eye and plasma after intravitreal administration of ranibizumab, a 48 kDa Fab monoclonal antibody (mAb) fragment, were obtained from literature. Mechanistically extended ocular models were developed for each species, including vitreous, aqueous, retina, plasma, and peripheral compartments.

The models were able to describe PK data in rabbit, monkey, and human. The diffusion rate from vitreous to aqueous declined with increasing species size. Aqueous clearance in human was estimated at a lower rate than assumed in literature. Retinal segment permeabilities were successfully translated from rabbit to monkey. Species-specific systemic parameters are necessary to describe the corresponding plasma PK.

This work enhances our understanding of the differences and similarities in large molecule ocular PK among different species. Inter-species disparities in diffusion through the vitreous humor, as well as in systemic drug disposition, were found. This emphasizes the need for additional research on physical and physiological processes underlying ocular drug diffusion and determining plasma PK, to gain a deeper understanding of these processes and to support future modeling and simulation of ocular therapeutics during drug development.

Introduction

Diseases of the retina can cause visual impairment and even lead to blindness. Common retinal diseases include age-related macular degeneration, diabetic retinopathy and retinal vein occlusion. Age-related macular degeneration (AMD), the most common cause for severe vision loss, is initially characterized by the development of drusen and changes in the retinal pigment. In the late stage (wet or neovascular AMD), newly developed leaky blood vessels cause fluid influx into the posterior eye tissue, damaging retinal structures (Mitchell et al., 2018). Diabetic retinopathy is a retinal disease arising from diabetes, where an increased permeability of the retinal blood vessels leads to diabetic macular edemas (DMEs), with fluid accumulation in the retina, causing retinal thickening (Musat et al., 2015). Retinal vein occlusion (RVO) is a group of disorders where the retinal vein reflux is impaired (Ip and Hendrick, 2019). Comparing these different diseases, a pathophysiological change in the ocular blood vessels is present in all three of them (Penn et al., 2008). The standard approach for treatment involves the use of antibodies (or their fragments) directed against vascular endothelial growth factor (VEGF) (Amo et al., 2017), such as ranibizumab, a Fab monoclonal antibody fragment. Intravitreal anti-VEGF therapy has been successfully applied for the treatment of wet AMD and has markedly decreased the worldwide prevalence of visual impairment (Mitchell et al., 2018).

To describe the disposition of large molecule drugs following intravitreal injection, mechanistic models can be applied that integrate physiological information to predict ocular half-life and drug exposure at the target site. The use of physiologically interpretable parameters in these models provides the basis for translation of the models between species. Cross-species translation is important for ocular PK studies, as sampling from posterior eye tissues is not possible in humans, and thus, modeling relies on information from animal data. Relevant pre-clinical species for ocular studies include rabbits and monkeys (Grossniklaus et al., 2010; Mage et al., 2019). So far, several ocular PK models for large molecules have been published. Hutton-Smith et al. (Hutton-Smith et al., 2017, 2018) developed a 3-compartment model describing ocular PK of several biologics in rabbits and translated it to describe ranibizumab ocular PK in human. Naware et al. (Naware et al., 2023) and Bussing and Shah (Bussing and Sha, 2020) presented whole-body PBPK models with detailed

ocular compartments for large molecules in rabbit, monkey, and human. Finally, we recently developed an extended ocular model for bevacizumab in rabbits, including injected eye, fellow eye and systemic compartments.

The aim of this study was to examine ocular PK within rabbit, monkey and human. Therefore, we used the previously developed extended ocular model structure to build a model for ranibizumab PK in rabbits and to translate it to monkey and finally to human. The increased understanding of ocular PK across species gained from this work is important to support ocular drug development.

Results

In this study, mechanistic extended ocular models were developed for rabbit, monkey and human. PK measurements from rabbit, monkey and human eye and plasma after intravitreal administration of ranibizumab were obtained from literature (see Table 4).

Table 4. PK data of ranibizumab used for model building.

Organism	Reported dose (adjusted dose*) [mg]	Number of individuals	Compartments with observations	Source
New Zealand White rabbit	0.3 or 3.0, bilateral	28	Vitreous, aqueous, retina, plasma	Kim et al., 2020
Dutch Belted rabbit	0.5 (0.55), unilateral	20	Vitreous, aqueous	Bakri et al., 2007
Cynomolgus monkey	0.5 or 2.0 (1.8), bilateral	12	Vitreous, aqueous, retina, plasma	Gaudreault et al., 2005

Cynomolgus monkey	0.5 (0.4), unilateral	3	Aqueous	Niwa et al., 2015
AMD, DME and RVO patients	0.5, unilateral	18	Aqueous	Krohne et al., 2012
AMD patients	0.5,	20	Plasma	Avery et al., 2017
DME patients	0.3,	15		
RVO patients	0.5,	18		
	one dose per month for three months, unilateral			

*adjusted to fit observations in injected vitreous

Extended ocular PK models were built in R, Version 4.2.2. First, the rabbit model was developed. In a next step, this was translated to monkey and, finally, to human. Concentration-time profiles were simulated with the rxode2 package, and the model was validated by comparison of the predicted profiles to the experimental observations. Simulations were performed showing the drug concentrations in the modeled individual, as well as a two-fold deviation range (indicated by dotted lines in the presented figures), considering the generally high variability of samples from ocular studies (Abduljalil et al., 2014; Yuan et al., 2022).

The model structure was described in detail in Chapter 3. Incorporating the basics of the Hutton-Smith et al. model (Hutton-Smith et al., 2017), it includes the injected eye vitreous, aqueous and retina, extended with a plasma compartment and a peripheral compartment (see Figure 18). The fellow eye was not included in this model structure as no observed data were available for the fellow eye compartments and an addition of the fellow eye would not have had any impact on the other compartments.

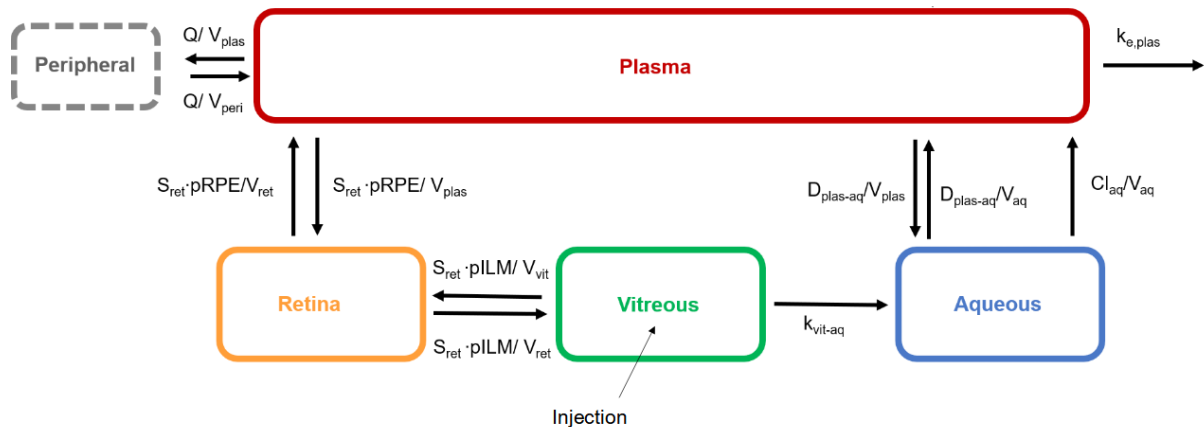


Figure 18. Schematic representation of ocular and systemic PK processes. Drug diffuses from the injection site at the center of the vitreous into aqueous or retina, and subsequently into plasma. From there it distributes into the peripheral compartment, gets eliminated, or distributes back into the eye. Drug transfer pathways are indicated by arrows. Cl_{aq} : aqueous humor clearance, $D_{plas-aq}$: transfer rate between plasma and aqueous chamber, $k_{e,plas}$: plasma elimination rate constant, k_{vit-aq} : transfer rate constant from vitreous to aqueous chamber, $pILM$: permeability of the inner limiting membrane, $pRPE$: permeability of the retinal pigment epithelium, Q : distribution rate between plasma and peripheral compartment, S_{ret} : retinal surface area, V_{aq} : volume of aqueous, V_{peri} : volume of peripheral compartment, V_{plas} : volume of plasma, V_{ret} : volume of retina, V_{vit} : volume of vitreous.

The model was built under several assumptions: At time point $t = 0$, the drug is injected into the vitreous with volume V_{vit} , resulting in an initial concentration of $dose/V_{vit}$ in the injected vitreous. The drug can diffuse from the vitreous to the aqueous chamber in a unidirectional manner. From there, it is eliminated via Schlemm's canal and reaches the systemic circulation with a clearance Cl_{aq} . The drug could also move between the vitreous and the retina by permeating the inner limiting membrane (ILM). As large molecules are typically not able to enter cells, the drug can access only a fraction of the entire retinal tissue volume (f_{ret}). From the retina, the drug can passively diffuse through the retinal pigment epithelium (RPE) and enter the systemic circulation through the fenestrated choroidal blood vessels (Amo et al.). The drug can also re-

enter the retina from the systemic circulation. From the plasma compartment, the drug distributes into the peripheral compartment with a flow rate Q . The drug is degraded from the plasma compartment with an elimination rate constant $k_{e,plas}$, which represents the sum of degradation in endosomes, renal clearance, and elimination upon target binding followed by drug-target-complex internalization and degradation. Furthermore, drug can flow from plasma to aqueous and vice-versa by passing the ciliary body with a transfer rate $D_{plas-aq}$ (Smith et al., 1975; Bill et al., 1980). Target binding in the ocular compartments is assumed to be negligible due to saturation of the low VEGF concentrations in the eye (Zhao et al., 2010). The described processes follow first-order kinetics and are represented by arrows with flow rate constants in Figure 18. The model equations can be found in Chapter 3.

Parameter values for ocular compartment volumes and plasma volumes were obtained from literature (Gaudreault et al., 2005; Feher, 2012; Missel, 2012; Gadkar et al., 2015). Retinal surface areas and retinal tissue volumes were calculated as described in Hutton-Smith et al. (Hutton-Smith et al., 2017), assuming the vitreous to be a sphere, partially covered by the retina. Further assuming azimuthal symmetry around the normal to the lens, the percentage of the vitreous in contact with the retina, Δ , was calculated by the formula

$$\Delta = 0.5 \cdot \left(1 - \cos \frac{L}{r_{vit}} \right)$$

with the cross-sectional arc length of the retina L , estimated from the scale diagrams presented in Missel et al. (Missel, 2012) and the vitreal radius r_{vit} , calculated from the vitreous volume. S_{ret} and V_{ret} were then approximated by the formulas

$$S_{ret} = \Delta \cdot 4\pi r_{vit}^2$$

$$V_{ret} = \Delta \cdot \frac{4}{3}\pi [(r_{vit} + \varepsilon)^3 - r_{vit}^3]$$

with the retinal thickness ϵ taken as reported by Missel et al. (Missel, 2012). The parameters used for estimation of S_{ret} and V_{ret} in the three species are summarized in Table 5.

Table 5. Parameters used for approximation of S_{ret} and V_{ret} .

	Rabbit	Monkey	Human	Description	Source
L [cm]	1.385	1.47	2.37	Cross-sectional arc length of the retina	Hutton-Smith et al., 2017, 2018; Missel et al., 2012
r_{vit} [cm]	0.713	0.803	1.02	Vitreous radius	Calculated from vitreous volume (Missel et al., 2012)
ϵ [cm]	0.010	0.020	0.022	Retinal thickness	Missel et al., 2012

In the rabbit and monkey models, Cl_{aq} was assumed to be equal to the aqueous humor production rate (Missel et al., 2012). For the human model, Cl_{aq} had to be estimated to describe the observed data well. The value of $p\text{RPE}$ was taken from Terasaki et al. (Terasaki et al., 2015), who measured ranibizumab permeability through highly polarized porcine RPE cells. From their published figure of permeated concentrations over time, we calculated a $p\text{RPE}$ of $0.018 \cdot 10^{-7} \text{ cm} \cdot \text{s}^{-1}$. The value of $p\text{ILM}$ was fixed to the value estimated for a Fab compound (Hutton-Smith et al. 2017), and f_{ret} was taken from our earlier developed bevacizumab model, assuming that the accessible retinal tissue volume fraction is similar for all large molecules. $D_{\text{plas-aq}}$ was set to a value 3-times higher than estimated for bevacizumab (see **Chapter 3**), assuming a linear correlation of $D_{\text{plas-aq}}$ with molecular weight in the absence of other evidence. The remaining parameters ($k_{\text{vit-aq}}$, $k_{\text{e,plas}}$, V_{peri} , Q) were estimated from the data, separately for each species. When necessary, the reported doses were adjusted (data sets of Bakri et al., 2007; Gaudreault et al., 2005; and Niwa et al., 2015) to capture the observed profile in the injected vitreous compartment.

The model parameters were either fixed from literature or estimated from the various data sets presented in Table 4. First, the rabbit model was developed, which was then translated to monkey, and finally to human. Table 6 summarizes the fixed and estimated parameter values of the three developed models.

Table 6. Parameter values of the bevacizumab ocular PK models.

Parameter [unit]	Rabbit model	Monkey model	Human model	Source	Description
V_{vit} [ml]	1.52	2.17	4.50	Missel et al., 2012	Volume of vitreous
V_{aq} [ml]	0.325	0.134	0.160	Rabbit, Monkey: Missel et al., 2012 Human: Hutton- Smith et al., 2018	Volume of aqueous chamber
V_{ret} [ml]	0.044	0.104	0.220	Missel et al., 2012	Volume of retina
S_{ret} [cm ²]	4.35	5.09	9.71	Rabbit: Hutton- Smith et al., 2017 Monkey: calculated according to Hutton-Smith et al., 2017	Retinal surface area

				Human: Hutton-Smith et al., 2018	
Cl_{aq} [ml • day ⁻¹]	4.32	2.45	0.90	Rabbit, Monkey: Missel et al., 2012 Human: Estimated	Aqueous humor clearance
V_{plas} [ml]	80	126	2,900	Rabbit: Gadkar et al., 2015 Monkey: Gaudreault et al., 2005 Human: Feher et al., 2012	Volume of plasma compartment
V_{peri} [ml]	300,000	400,000	8,000,000	Estimated	Volume of peripheral compartment
Q [ml • day ⁻¹]	30,000	2,000	1,000	Estimated	Distribution rate between plasma and peripheral compartment
$k_{e,plas}$ [day ⁻¹]	150	10.0	7.00	Estimated	Plasma elimination rate constant
k_{vit-aq} [day ⁻¹]	0.260	0.250	0.120	Estimated	Diffusion rate constant from

					vitreous to aqueous chamber
pILM [$\text{cm} \cdot \text{s}^{-1}$]	$1.88 \cdot 10^{-7}$	$1.88 \cdot 10^{-7}$	$1.88 \cdot 10^{-7}$	Hutton-Smith et al., 2017 (reported value for Fab)	Permeability of the inner limiting membrane (ILM)
pRPE [$\text{cm} \cdot \text{s}^{-1}$]	$1.80 \cdot 10^{-8}$	$1.80 \cdot 10^{-8}$	$1.80 \cdot 10^{-8}$	Terasaki et al., 2015 (in-vitro)	Permeability of the retinal pigment epithelium (RPE)
f_{ret}	0.41	0.41	0.41	Taken from fellow eye model (rabbit bevacizumab)	Retinal volume fraction accessible to large molecule drug
$D_{\text{plas-aq}}$ [$\text{ml} \cdot \text{day}^{-1}$]	0.06	0.06	0.06	Fixed to 3-times higher value than for bevacizumab	Transfer rate between plasma and aqueous chamber

The model performance applying these parameter values is documented in Figures 19-21. The presented rabbit model describes the observed data in the ocular compartments and plasma reasonably well, with the last measured concentration of the highest dose being underpredicted in aqueous and retina (see Figure 19).

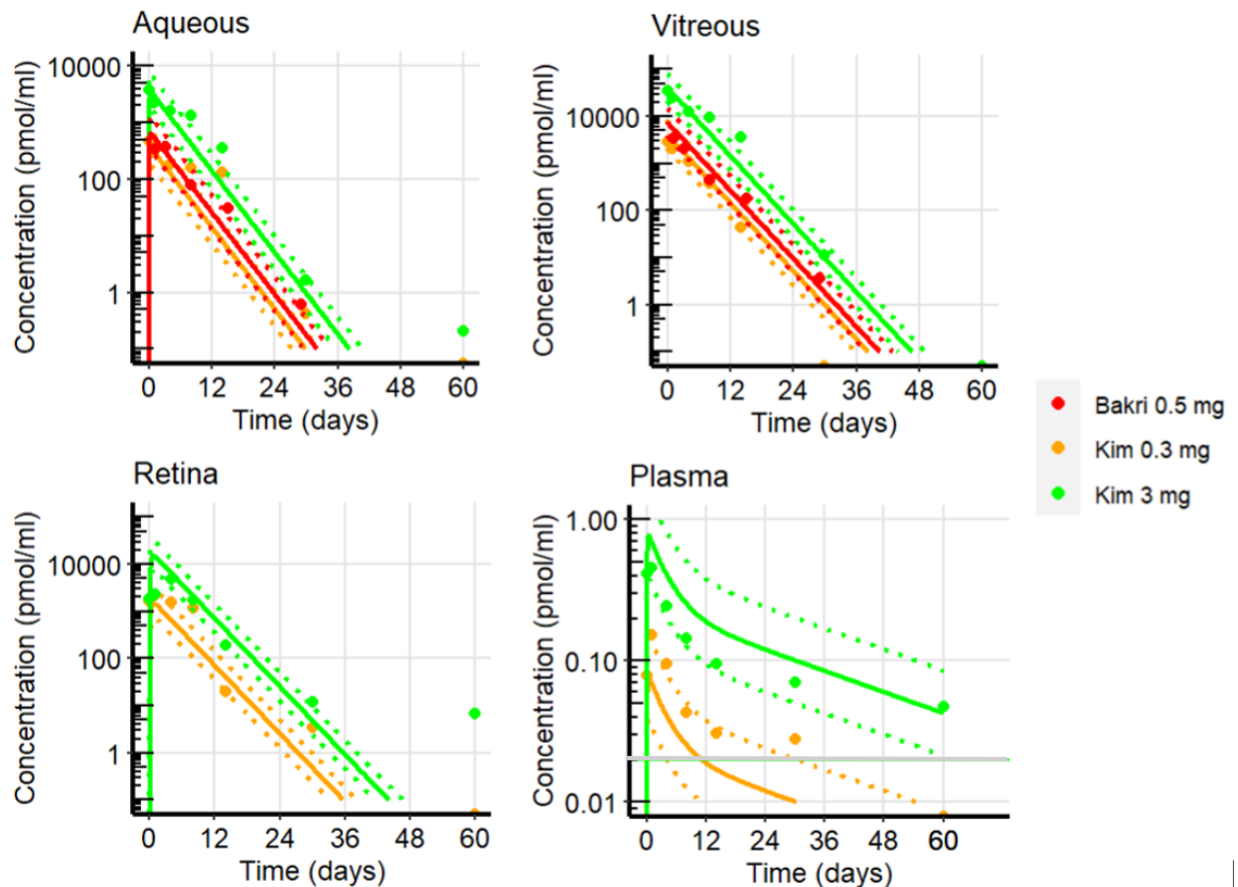


Figure 19. Rabbit ocular ranibizumab PK model. Shown are free ranibizumab concentrations over time in aqueous, vitreous, retina and plasma compartments. Solid lines: simulations, with corresponding dotted lines: 0.5-fold and 2-fold deviation. Red points: observed data 0.5 mg dose (Bakri et al., 2007), orange points: observed data 0.3 mg dose (Kim et al., 2020), green points: observed data 3 mg dose (Kim et al., 2020), gray horizontal line: lower limit of quantification (Kim et al., 2020). Data points on the x-axes refer to measurements below the lower limit of quantification.

The presented monkey model describes the observed data in the ocular compartments and plasma well (see Figure 20). The pILM and pRPE were taken from the rabbit model, assuming that these values are conserved across species. The observed retinal concentrations are slightly overpredicted, however, the retinal samples from monkeys included retina, RPE, Bruch's membrane, choroidal blood vessels and Ames' medium. Therefore, these observations are expected to be lower than the actual retinal concentrations.

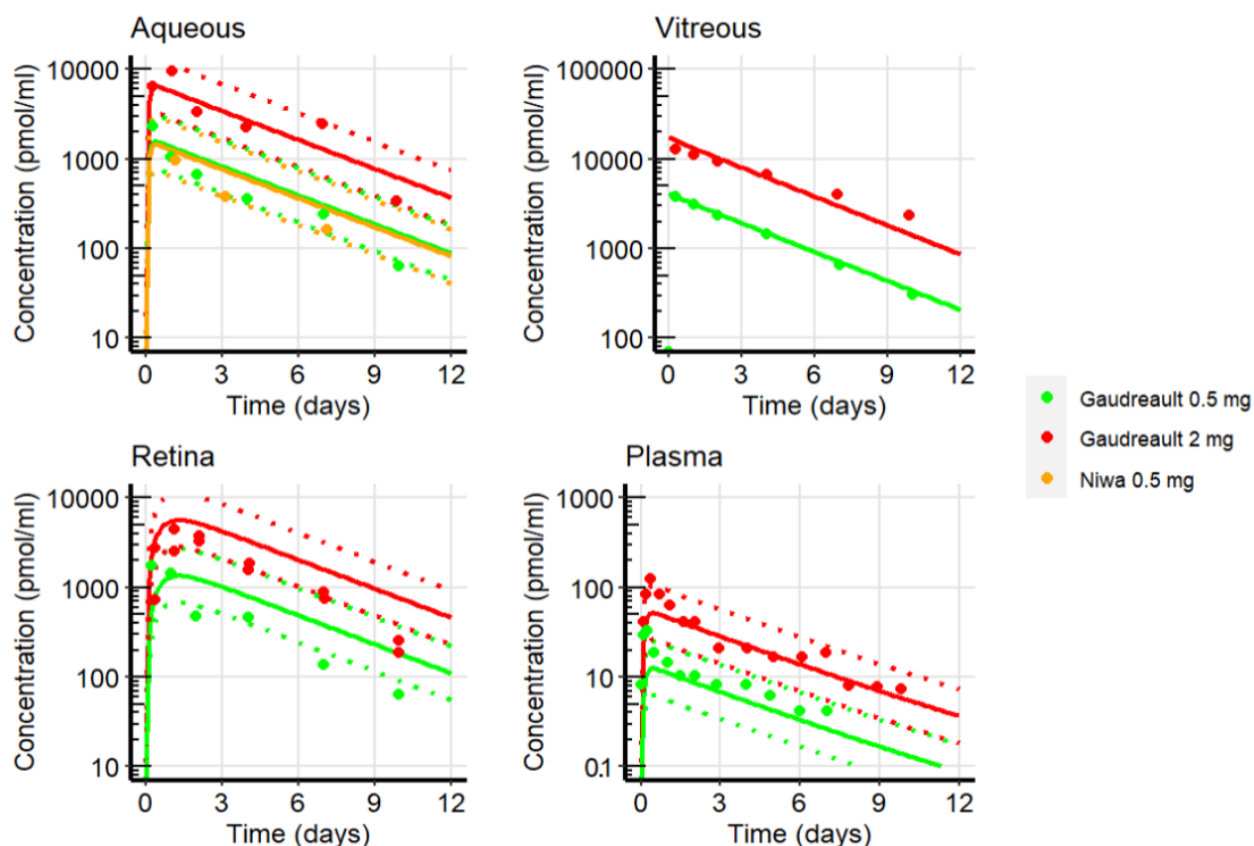


Figure 20. Monkey ocular ranibizumab PK model. Shown are free ranibizumab concentrations over time in aqueous, vitreous, retina and plasma compartments. Solid lines: simulations, with corresponding dotted lines: 0.5-fold and 2-fold deviation. Green points: observed data 0.5 mg dose (Gaudreault et al., 2005), red points: observed data 2 mg dose (Gaudreault et al., 2005), orange points: observed data 0.5 mg dose (Niwa et al., 2015).

The presented human model describes the observed data in aqueous and plasma well (see Figure 21). The pILM and pRPE were taken from the rabbit model, assuming that these values are conserved across species.

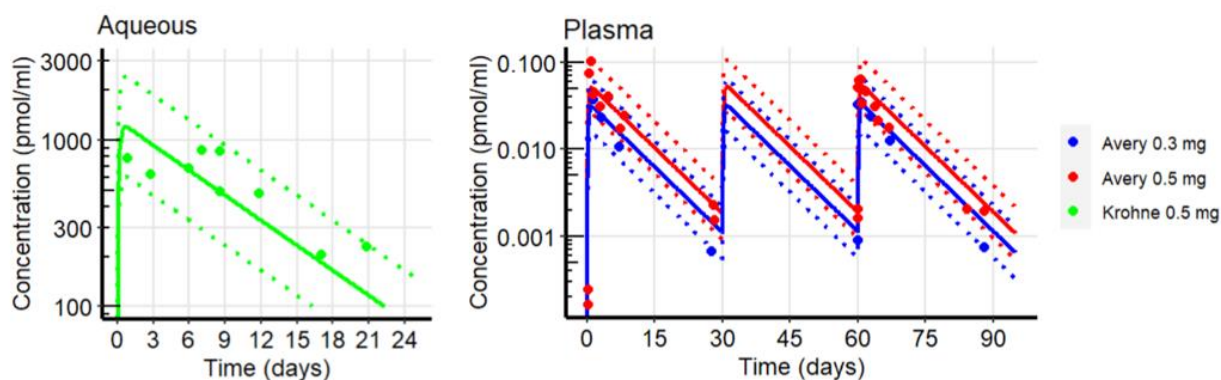


Figure 21. Human ocular ranibizumab PK model. Shown are free ranibizumab concentrations over time in aqueous and plasma compartments. Solid lines: simulations, with corresponding dotted lines: 0.5-fold and 2-fold deviation. Blue points: observed data 0.3 mg dose (Avery et al., 2017), red points: observed data 0.5 mg dose (Avery et al., 2017), green points: observed data 0.5 mg dose (Krohne et al., 2012).

Discussion

The presented newly developed models offer the opportunity to compare physiological parameters that impact ocular PK between the investigated species. We applied the refined model structure that we developed previously for the IgG bevacizumab in rabbit, and which was validated with ocular data from injected and fellow eyes, as well as plasma data (see **Results of Chapter 3**). We showed here that this model structure is applicable also for the Fab ranibizumab, in rabbit, monkey and human. Compared to literature models that consider the eye as an isolated organ, the proposed model structure offers considerable benefits for clinical application by including a plasma compartment. This extension allows for ocular predictions based on plasma data, which clinical studies are often limited to. Furthermore, the model extension facilitates the assessment of potential systemic toxicity of ivt administered drugs.

Vitreous diffusion

The estimated k_{vit-aq} was fastest for rabbit, followed by monkey and human values (0.26, 0.25 and 0.12 day⁻¹, respectively). For different IgG mAbs, Naware et al.

(Naware et al., 2023) report flow rates from vitreous to aqueous of $8.00 \cdot 10^{-6} \text{ L} \cdot \text{h}^{-1}$ in rabbits, $1.64 \cdot 10^{-5} \text{ L} \cdot \text{h}^{-1}$ in monkeys and $4.08 \cdot 10^{-5} \text{ L} \cdot \text{h}^{-1}$ in humans, which corresponds to k_{vit-aq} values of 0.13, 0.19 and 0.24 day^{-1} , respectively (considering V_{vit} rabbit, monkey and human as 1.52 ml, 2.04 ml and 4.00 ml, respectively, as Naware et al. (Naware et al., 2023) assumed in their models). In consequence, they estimated the fastest diffusion in human, followed by monkey and rabbit, reporting a different trend between these three species. However, their model structure includes a backflow from aqueous into vitreous, thus, the values cannot be directly compared to our model. Hutton-Smith et al. (Hutton-Smith et al., 2017) estimated a slower diffusion for a Fab (50 kDa) in rabbits compared to our estimation for ranibizumab. They report a k_{vit-aq} of 0.197 day^{-1} . The molecular weight of 50 kDa is slightly larger than that of ranibizumab (48 kDa), so a slower diffusion is reasonable.

Hutton-Smith et al. (Hutton-Smith et al., 2016) proposed a calculation method to derive k_{vit-aq} for different species and molecules. In their publication they used the following formula:

$$k_{vit-aq} = \frac{S^*}{S} \cdot \frac{6k_B T}{6\pi\eta R_{hyd} r_{vit}^2}$$

with k_{vit-aq} : vitreous to aqueous diffusion rate constant, S^* : part of the vitreal surface area through which a molecule can transfer to the aqueous chamber, S : total vitreal surface area, k_B : Boltzmann constant, T : absolute temperature, η : viscosity of physiological saline (0.15 M NaCl, at 37°C), R_{hyd} : hydrodynamic radius of the drug and r_{vit} : vitreal radius.

According to this formula, the diffusion towards the anterior chamber is faster for smaller molecules and in species with a smaller vitreous body and a larger vitreous-aqueous interface. To see how well this formula applies to the current model, we compared the calculated values with our model estimates (see Table 7). S^*/S for every species was taken as 0.17 from Hutton-Smith et al. (Hutton-Smith et al., 2016) (mean value, based on the anatomically accurate models of the eye given by Missel et al., 2012). The vitreal radius was calculated from the vitreal volume, assuming the vitreous as a sphere. The hydrodynamic radius for different molecules was calculated according to Hutton-Smith et al. (Hutton-Smith et al., 2016):

$$R_{hyd} = \left(\frac{3vMW}{4\pi N_A} \right)^{1/3}$$

with MW: molecular weight, N_A : Avogadro's number and v : partial specific volume of protein, taken as 0.73 cm³/g (Harpaz et al., 1994).

Table 7. Estimated and calculated values for k_{vit-aq} of ranibizumab.

	Rabbit	Monkey	Human
k_{vit-aq} model estimates [day⁻¹]	0.26	0.25	0.12
k_{vit-aq} calculated [day⁻¹]	0.23	0.18	0.11

In contrast to the model estimates of Naware et al. (Naware et al., 2023), the species trend of our k_{vit-aq} model estimates follows the trend of the calculated k_{vit-aq} . Diffusion is fastest in rabbit, followed by monkey and human, which is physiologically plausible due to the growing vitreal volume. For rabbit, monkey and human, the estimated diffusion is slightly faster than calculated with the Hutton-Smith formula (rabbit: 12%, monkey: 28% and human: 9%). Notably, their formula for k_{vit-aq} depends on parameters which exhibit certain variation. For instance, the vitreal volume, and thus, the vitreal radius, varies between individuals of the same species. To assess this variability, we calculated vitreal radii from the lowest and highest values of V_{vit} found in literature, as summarized in Table 8.

Table 8. Vitreal parameters for calculation of ranges for k_{vit-aq} .

	Rabbit	Monkey	Human	Source
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V_{vit} [ml] lowest value; highest value	0.55; 1.80	1.71; 2.17	2.50; 10.0	Rabbit: (Atsumi et al., 2015) Monkey: (Missel et al., 2012; Rasmussen et al., 2007) Human: (Borkenstein et al., 2024)
r_{vit} [cm] lowest value; highest value	0.50; 0.75	0.74; 0.80	0.84; 1.34	Calculated from V _{vit}

Another source of variability in k_{vit-aq} is the vitreal viscosity η . The proposed formula uses the viscosity of physiological saline, which differs from in-vivo conditions. Additionally, inter-species and inter-individual differences in vitreal viscosity have been observed experimentally ex-vivo. Inter-individual variability was reported for bovine eyes (7.5%) and porcine eyes (80%) (Shafaie et al., 2018). No literature was found examining inter-individual viscosity differences specifically for rabbits, monkeys or humans. Figure 22 shows the k_{vit-aq} model estimates within the calculated k_{vit-aq} parameter ranges. The variability of r_{vit} was taken as in Table 8, and a 20% deviation was allowed for η (given the large differences between bovine and porcine eyes, this value was chosen arbitrarily with the aim to illustrate the sensitivity of the calculated k_{vit-aq} to variations in viscosity).

Applying these assumptions, the estimated values of k_{vit-aq} are within the calculated ranges. Thus, the formulas suggested by Hutton-Smith et al. (Hutton-Smith et al., 2016) may be applicable, however, it must be kept in mind that inter-individual variability in vitreous size and viscosity influences vitreal diffusion. If k_{vit-aq} is calculated and used as model input, it would thus be recommended to use a precise value of the individual vitreous dimensions for calculation. Individual vitreal viscosity values would

also be valuable but are not feasible to measure. However, note that the actual inter-individual variability of η might be even larger than assumed here.

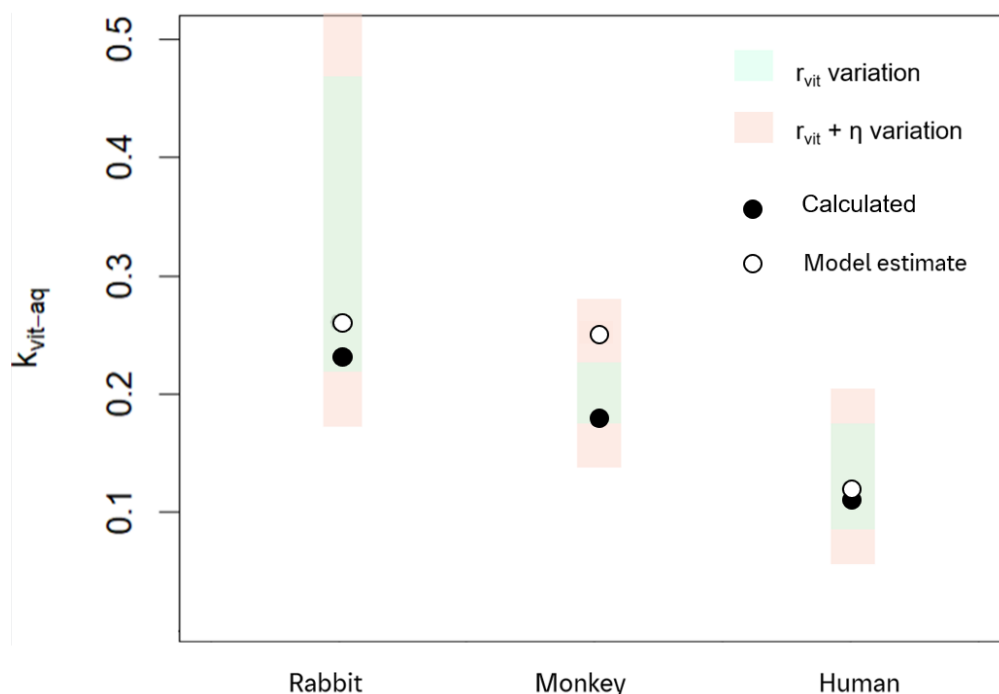


Figure 22. k_{vit-aq} model estimates compared to the calculated k_{vit-aq} parameter ranges. Empty circles: model estimates of k_{vit-aq} , filled circles: calculated k_{vit-aq} . Green bars: calculated k_{vit-aq} given variability in r_{vit} , orange bars: calculated k_{vit-aq} given variability in r_{vit} and 20% variation in η .

Retinal PK

The measured ranibizumab concentrations in rabbit after 60 days in aqueous and retina are underpredicted, showing a right curvature in the concentration profiles at the highest dose of 3 mg (Figure 19). For the lower doses and the vitreous, the observed concentrations after 60 days are not reported. The observed plasma concentration after 3 mg at 60 days is well described.

With the current study, we were able to show that rabbit and monkey retinal concentrations can be described with the same parameter set for $pRPE$, $pILM$ and f_{ret} . Unfortunately, for humans the only available observed data are from aqueous and plasma sampling, so we are not able to evaluate our retinal model predictions to date. It is reasonable to assume that these parameters can also be transferred from

monkeys to healthy humans, which would be an important information for clinical studies. Regarding the fraction of large molecules that are eliminated via the posterior route after intravitreal injection, there are estimates for individual compounds (Amo et al., 2017), but to the best of our knowledge there is no study so far that investigated the relationship between molecular weight and fraction eliminated via the posterior route. Experimental data on Cl_{aq} , pILM, pRPE and f_{ret} would be very valuable as input parameters. Alternatively, additional modeling and simulation studies like the current one, which estimate these parameters, would be helpful to find a trend to determine missing parameters from drug properties and ultimately facilitate predictions of retinal PK in humans.

Aqueous clearance

Similar to the retinal compartment, the 60-day observation in the rabbit aqueous humor (highest dose only) is underpredicted, while the corresponding observation of the lower dose of Kim et al. was below the limit of quantification. In the monkey model, the highest dose observations in the aqueous are underpredicted, however the observed aqueous humor concentrations following the two lower dose applications are predicted well. For rabbit and monkey, an aqueous clearance equal to the literature value for aqueous humor production rate was assumed (Missel et al., 2012). For the human model, the respective value of $3.6 \text{ ml} \cdot \text{day}^{-1}$ (Missel et al., 2012) would lead to an underprediction of the aqueous concentrations. Hutton-Smith et al. (Hutton-Smith et al., 2018) used this value when describing ranibizumab PK in humans. However, their model was validated using VEGF data in aqueous humor, and not with ranibizumab data. In our model, we show that a value of $0.9 \text{ ml} \cdot \text{day}^{-1}$ is necessary to adequately describe the aqueous observations. A possible explanation for a lower Cl_{aq} in human compared to the aqueous humor production rate, could be that we are examining patients (often of higher age) and not healthy humans. Various anatomical differences are conceivable. For instance, ocular diseases or aging may cause structural changes in the trabecular meshwork which is responsible for draining the aqueous humor into Schlemms' canal, which would result in a change in Cl_{aq} (Tektas and Lütjen-Drecoll, 2009). Also, in the presented model structure a unidirectional flow from vitreous to aqueous was assumed, due to the high flow rate of aqueous humor out of the aqueous chamber. However, this assumption might not hold true if part of the

aqueous humor is able to flow against this current. Adding a backflow from aqueous into vitreous would be a way to get a better fit of the aqueous concentrations without reducing Cl_{aq} , however, this would result in a larger estimate for k_{vit-aq} , which agrees less with the earlier described physical formula.

Transfer between plasma and aqueous

The rabbit aqueous concentration-time profile shows a right curvature at the latest time point and the highest dose, which could be explained by a back-diffusion from plasma to the aqueous chamber. As the pathway through Schlemms' canal is likely unidirectional, this backflow might rather occur through the ciliary body, as discussed in Chapter 3. For our earlier developed bevacizumab model, $D_{plas-aq}$ was estimated at 0.02 day^{-1} . As bevacizumab (150 kDa) is roughly 3-fold larger than ranibizumab (48 kDa), it is reasonable that the diffusion of ranibizumab can be faster. Thus, $D_{plas-aq}$ was set to $0.06 \text{ ml} \cdot \text{day}^{-1}$, 3-fold higher than estimated for bevacizumab, assuming a linear relationship in the absence of other evidence. The aqueous simulation displays a right curvature at late time points (not shown), however, the latest observation of the highest dose is still underpredicted (as for the retinal profile). It should be taken into consideration that this observed concentration at 60 days might be biased by below limit of quantification (BLQ) measurements. A better description would require a higher value for $D_{plas-aq}$, which does not seem physiologically plausible. Notably, the right curvature is seen only in rabbit data, where measurements were taken at extended time points up to 60 days. In the monkey and human models $D_{plas-aq}$ is not identifiable.

Plasma kinetics

In rabbits, the plasma profile was slightly overpredicted for the high dose, and slightly underpredicted for the low dose (Figure 19). Note that the doses in Kim et al. (Kim et al., 2020) differ 10-fold, however, the plasma observations differ only about 3-fold, making it impossible for the presented model structure to perfectly capture both concentration-time profiles. While V_{plas} was fixed to literature values for rabbit, monkey and human, $k_{e,plas}$, V_{peri} and Q were estimated. The estimates for V_{peri} are in a similar relation as V_{plas} across species. The estimated value for Q was highest for rabbit, followed by monkey and human. Assuming a distribution of ranibizumab through pores in the blood vessels (Niederalt et al., 2018), a faster distribution in

smaller species is not reasonable. An alternative explanation might be the higher heart rate in smaller animal species, resulting in faster distribution of perfusion-limited compounds (Milani-Nejad and Janssen, 2014). In the current model, $k_{e,plasma}$ combines all relevant systemic elimination processes of ranibizumab, including the endosomal degradation in endothelial cells, renal clearance, as well as indirect processes via binding to VEGF and subsequent degradation of the drug-target-complex. With respect to renal clearance, a higher elimination rate constant (expressed in day^{-1}) in smaller species is reasonable, due to their smaller plasma volume. For rabbit and human, there are no literature data of ranibizumab systemic clearance. Regarding monkeys, our estimated value is higher than the one reported by Gaudreault et al. (Gaudreault et al., 2005), who determined ranibizumab clearance using the plasma concentration-time course after iv administration. As the resulting plasma levels after iv administration are higher than following ivt administration, the role of target-mediated drug disposition (TMDD) likely differs in the two scenarios. Bevacizumab, as an anti-VEGF agent with lower binding affinity compared to ranibizumab (Papadopoulos et al., 2012), shows typical TMDD PK in plasma at low iv doses (Basu et al., 2020). Thus, for the low ranibizumab concentrations after ivt administration, it is likely that target binding accelerates ranibizumab elimination, which is reflected by a higher $k_{e,plasma}$ in our model. These circumstances show the value of plasma PK data after iv administration, especially using low doses. Plasma sampling after ivt administration alone is not enough to inform the complex TMDD PK, while there are knowledge gaps regarding the blood-ocular barrier. Observed profiles which exhibit the typical TMDD shape could be used to estimate relevant parameters for target abundance, target turnover and target binding kinetics, and to subsequently determine inter-species differences. For clinical scenarios, where ocular predictions often rely on plasma data, information about systemic PK parameters would be valuable to draw conclusions for ocular PK.

When comparing the presented models for animals and humans, it must be kept in mind that human PK data was not obtained from healthy subjects, but from patients with different retinal diseases. Their anatomy might differ from healthy people, for instance they might show a lower aqueous clearance. The same applies to the retinal segment permeabilities. We assume here, that human pILM and pRPE do not differ

from monkey and rabbit parameters, however, changes in the posterior eye due to retinal diseases were reported, e.g. leaky blood vessels and disruption of the RPE structure, which might impact pILM and/or pRPE (Amo et al., 2017). Unfortunately, as there is no retinal data available from humans, these parameters could not be estimated and had to be taken from animal models or in-vitro data.

To conclude, this study contributes to a better understanding of the inter-species similarities and differences in ocular PK. Parameters which can be translated between rabbit and monkey were identified, and may also be applied to human. This work shows differences between species in vitreal diffusion and highlights several points where further research is needed to better understand these differences. Furthermore, the presented results demonstrate the relevance of a more detailed model structure including the representation of systemic PK for ocular predictions from sparse data. Overall, the developed models improve our understanding of ocular PK translation between species.

Chapter 5: General conclusion and future work

In conclusion, this dissertation advances the field of ocular pharmacokinetics (PK) by enhancing our understanding of the complex processes governing the distribution and elimination of large molecules in the eye across different species. By employing PBPK modeling and mechanistic PK modeling as tools, this research addresses critical knowledge gaps in ocular physiology, exposure at the target site, and whole-body cross-species translational predictions. The results presented in this dissertation highlight the utility of mechanistic models in elucidating ocular PK dynamics, even when direct sampling from human ocular tissues is not feasible. This work also demonstrates how considering both ocular and systemic PK within a unified framework improves our understanding of the blood-ocular barrier and enables more reliable ocular drug exposure predictions based on limited clinical data, such as plasma concentrations.

A central aim of this dissertation was to tackle the challenges posed by the limited understanding of ocular permeability barriers and their impact on drug exposure following intravitreal (ivt) administration of large molecule drugs. One of the main difficulties in drug development for ocular indications arises from the absence of direct retinal sampling in humans, necessitating indirect approaches to describe the drug behavior at the target site. The models developed in this dissertation successfully address these issues by incorporating physiological differences between species and by linking ocular PK to systemic PK. This integrative approach enhanced our understanding of the dynamics at the blood-ocular barrier and brought us closer to our future goal of predicting drug exposure in the retina using plasma PK data alone.

In Chapter 2, a case study on efalizumab demonstrates how whole-body PBPK models can effectively capture inter-species differences in systemic large molecule PK following iv administration. By comparing the target binding and elimination parameters between rabbits, non-human primates (NHPs), and humans, the study identified the cross-species translatability of key parameters, such as target turnover and drug-target-complex internalization. The results further showed, that while parameters related to target-mediated drug disposition (TMDD) could be translated from NHPs to humans, parameters related to FcRn affinity could not. Such findings deepen our understanding of inter-species PK similarities and differences and pave

the way for more precise and reliable predictions of drug behavior for first in human studies.

Chapter 3 shifts focus to ocular PK of large molecule drugs following ivt administration in rabbits, the species with the most data from different ocular tissues available. An extended ocular model structure was developed to include not only the injected eye but also systemic compartments and the fellow uninjected eye. This refined model incorporates new parameters that were not previously analyzed, allowing for a more detailed representation of ocular PK. The study used bevacizumab as tool compound to simulate concentration-time profiles in the injected eye, systemic circulation, and fellow eye, shedding light on the processes influencing ocular drug distribution and the bidirectional exchange between the eye and systemic circulation.

One of the significant achievements of this extended model is its ability to estimate the volume fraction of the retina accessible to bevacizumab and to refine the estimated value of the RPE permeability. By aligning the estimated RPE permeability with in-vitro experimental values, the model offers a more physiologically plausible representation of the retinal compartment, improving predictions of drug exposure at the target site. The introduction of a backflow mechanism from the systemic plasma to the aqueous chamber enhances the model's accuracy in predicting drug concentrations in the fellow eye, providing new insights into the blood-ocular barrier. Collectively, this refined model structure contributes to a more comprehensive understanding of the fundamentals underlying both ocular and systemic PK following ivt administration.

Building on the understanding of Chapter 2 and 3, Chapter 4 focuses on ocular PK cross-species translation, which is crucial for clinical studies due to the inherent limitations of sampling from posterior eye tissues in humans. This study examines the PK of ranibizumab in rabbits, monkeys, and humans after ivt administration, developing mechanistic extended ocular models for each species. The models describe key compartments, such as the vitreous, aqueous, retina, plasma, and peripheral tissues, enabling a detailed comparison of drug behavior across species. The results show that the rate of diffusion from the vitreous to aqueous declines with

increasing species size, reflecting anatomical and physiological variations between species. They also indicate that aqueous clearance in humans is lower than previously assumed in literature.

One of the noteworthy findings of Chapter 4 is the successful translation of retinal segment permeabilities from rabbits to monkeys, showing the potential for cross-species extrapolation of certain parameters. However, the results also highlight the necessity of accounting for differences in systemic parameters across species to accurately describe plasma PK. This nuanced understanding of inter-species differences in ocular and systemic drug behavior emphasizes the importance of targeted research to bridge the current knowledge gaps and to improve the predictability of human ocular PK based on animal models.

The collective findings of this dissertation provide valuable insights into ocular PK and cross-species translation of large molecule modeling and simulation, developing improved models to support drug development of ocular therapeutics. The extended ocular model structure developed in Chapter 3, which incorporates the dynamics of the fellow eye, offers a more detailed representation of ocular PK and highlights the complex interactions between ocular tissues and systemic circulation. Compared to existing models in the literature that do not include plasma, peripheral, and fellow eye compartments, the structural extension of the model provides significant improvements that were validated using observed data from plasma and fellow eyes. This calibration of the model to measured data from tissues that had not been included for model development before, increases the confidence in the estimated model parameter values. The structural extensions support predictions based on plasma data and evaluation of systemic and fellow eye toxicity. In addition, the physiological representation of retinal volume and permeabilities makes the model suitable for physiologically based interpretation and future incorporation into a whole-body PBPK model.

Moving forward, the findings of this dissertation suggest several avenues for future research. Additional studies are needed to further refine the parameters governing retinal permeability and vitreal drug diffusion, as well as to explore the applicability of these models to other large molecules and therapeutic compounds. Incorporating

data from diverse animal species and clinical scenarios would help validate and generalize the models, ultimately supporting the translation of preclinical findings into clinical practice. Moreover, the development of comprehensive human PBPK models that decrease the need of animal testing remains a key goal, which can hopefully be achieved in the near future by integration of data on target expression and internalization rates.

In summary, this dissertation advances the field of ocular PK by improving our understanding of large molecule drug distribution and elimination in different species and by refining the modeling frameworks used to describe and translate these dynamics between rabbits, monkeys and humans. The developed PK models provide a valuable tool to bridge knowledge gaps related to ocular permeability barriers, systemic drug elimination, and inter-species differences. By offering a more detailed and physiologically accurate representation of large molecule drug ocular PK within a systemic context, this work enhances our ability to predict drug behavior in the eye, supports the development of safer and more effective ocular therapies, and contributes to the broader field of pharmacokinetic modeling and drug development.

Chapter 6: Material and Methods

Materials and Methods for chapter 2 partially published as:

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Contributions:

M. Franz created the table and wrote the chapter. N. Hanke, R. Jairam and L. Kuepfer revised the chapter.

Materials and Methods for chapter 3 contributions:

M. Franz wrote the majority of the chapter. Holger Fuchs wrote the section "In-vivo PK study" and Wolfgang Rist wrote the section "Bioanalytics". The chapter was revised by the co-authors Ravi Kumar Jairam, Anna-Kaisa Rimpelä, Guangda Ma, Wolfgang Rist, Holger Fuchs, Lars Kuepfer and Nina Hanke.

Materials and Methods for chapter 4 contributions:

M. Franz wrote the chapter. N. Hanke, R. Jairam and L. Kuepfer revised the chapter.

Materials and Methods for chapter 2

Data

Rabbit, NHP and human plasma PK data after intravenous administration of efalizumab were obtained from literature (see Table 9).

Table 9. PK data of efalizumab used for model building.

Species	Doses	Source
Human	0.1; 0.3; 1.0; 3.0; 10 mg/kg	Bauer et al., 1999
Chimpanzee	8 mg/kg	Bauer et al., 1999
New Zealand White Rabbit	2 mg/kg	Mould and Green, 2010

Mean data were digitized from the published figures with the WebPlotDigitizer tool (<https://automeris.io/WebPlotDigitizer/>). Data from Ng et al. (Ng et al., 2005) were not considered for model development as the concentration-time profile for the 0.6 mg/kg dose does not align well with the other observed data (see **Supplementary Figures**). Also, data from Gottlieb et al. (Gottlieb et al., 2000, 2002) were not used, as they only published data following multiple administration.

Model development

PBPK models for efalizumab (rabbit, NHP and human) were built with the Open Systems Pharmacology Suite (OSP Suite, <http://www.open-systems->

pharmacology.org), Version 11.1. Its software tool PK-Sim allows the selection of different species, accounting for anatomical and physiological differences between them, such as organ volumes, blood flow rates and tissue composition. We recently evaluated the applicability of the rabbit model structure (Jairam et al., 2024). The standard individuals were created with the following body weights: human (male, 73 kg), monkey (52.8 kg), rabbit (3 kg).

The model was fitted to the data using the Levenberg-Marquart algorithm in the OSP suite. The PBPK models were validated by comparison of the predicted concentration-time profiles to the experimental observations. The OSP Suite-R package was used to generate concentration-time plots (OSP suite results were imported via the ospsuite package).

Sensitivity analysis

Sensitivity analysis was performed for single parameters of the model (local sensitivity analysis). It provides information on how the variation of certain parameters contributes to the overall uncertainty of a model. The area under the curve (AUC) extrapolated to infinity was used as the PK parameter to compare sensitivities. Sensitivity was calculated as the ratio of the relative change of AUC and the relative variation of the input parameter according to the following equation:

$$S = \frac{\Delta AUC}{AUC} \cdot \frac{p}{\Delta p}$$

with AUC: simulated AUC with the original input parameter value, ΔAUC : change of simulated AUC, Δp : change of the input parameter value, p : input parameter value, and S : sensitivity.

Parameters related to target turnover, target-binding and FcRn-binding were selected for the analysis in order to quantify their relevance for cross-species translation. This included all estimated model parameters, as well as parameters which were assumed to have a strong influence on PK. Sensitivity was calculated in MoBi with a relative

parameter perturbation of 1000 % (variation range = 10, maximum number of steps = 9).

Materials and Methods for chapter 3

In-vivo PK study

Eight male New Zealand White rabbits (CrI:KBL(NZW), Charles River) weighing 2.2 – 2.4 kg were included into the study. For bilateral intravitreal injections animals were anaesthetized with medetomidine (0.2 mg/kg i.m.) and midazolam (1 mg/kg i.m.). In case nystagmus occurred, anaesthesia was deepened using propofol (i.v.) and topical anesthesia was performed by oxybuprocaine eye drops. The ocular surface was rinsed with povidone iodine solution (5%). After removal of excess disinfectant 50 µL Avastin® (Roche Pharma AG) were injected via a 0.3 mL insulin syringe (BD Microfine U-100 Insulin, 30G needle) resulting in a total dose of 1.25 mg bevacizumab per eye. GenTeal® (Alcon) lubricating eyedrops were applied and anaesthesia was administered by subcutaneous injection of 1 mg/kg atipamezole and 0.1 mg/kg flumazenil. In-vivo blood samples were taken from the lateral ear vein at 1, 2, 4, 8, 48, 72, and 504 hours after dosing and at the respective terminal time points 24, 168, 361, and 698 h. At each of these time points two animals were euthanized (0.2 mg/kg i.m. medetomidine and 1 mg/kg i.m. midazolam followed by an overdose of 128 mg pentobarbital i.v.) and ocular samples were taken. First, aqueous humor was sampled by paracentesis. Then, the eye was explanted and a circular incision about 2 mm lateral to the limbus was made. The vitreous body and the retina were sampled. Retina samples were transferred to pre-weighed Precellys® (Bertin Technologies) tubes for homogenization. The animal experiments were performed according to the German Animal Welfare Act and approved by the local authorities (Regierungspräsidium Tübingen, licence no. 15-012-G).

Bioanalysis

Avastin was quantified using a sandwich enzyme-linked immunosorbent assay (ELISA). The capturing antibody was a goat α -human IgG, monkey adsorbed, from SouthernBiotech (ID 2049-01) and the detection antibody was a sheep α -human IgG conjugated with HRP, monkey adsorbed, from The Binding Site (AP003.M). The assay execution and data analysis were performed as described by Boch et al. (Boch et al., 2022)

Data

Rabbit PK data after unilateral intravitreal administration of bevacizumab were obtained from literature. Mean data were digitized from published figures using the WebPlotDigitizer tool (<https://automeris.io/WebPlotDigitizer/>). In addition, new in-house measurements after bilateral intravitreal dosing were used for model development.

Model evaluation

The naïve pooled algorithm with a proportional error model was used as the estimation method. During the estimation procedure, different initial values were tested, and the best fit was selected according to the log-likelihood value and by comparing the simulations to the observed data.

To evaluate the quality of the base and final models, the absolute average fold error (AAFE) was calculated according to formula

$$10^{\frac{1}{n} \sum_1^n |\log(y'/y)|}$$

with the number of observations, n ; the predicted value, y' ; and the observed value, y .

A sensitivity analysis was performed for single model parameters (local sensitivity analysis), providing information on how the variation of a parameter contributes to the overall uncertainty of the model. The parameters f_{ret} , p_{ILM} and p_{RPE} were selected for the analysis to evaluate identifiability of retinal parameters before and after adjustment of the retinal volume. The area under the curve (AUC) from time 0 to 30 days was used as the PK parameter to compare sensitivities in the retinal compartment (using the whole retinal volume, including cells). Sensitivity was

calculated as the ratio of the relative change of retina AUC. The parameters pILM and pRPE were varied by a factor of 10 and f_{ret} was varied by a factor of 2, to not exceed values of 0 and 1 for the accessible retinal fraction.

Materials and Methods for chapter 4

Data

PK data of rabbit, monkey and human eye and plasma after intravitreal administration of ranibizumab were obtained from literature. Mean data were digitized from published figures with the WebPlotDigitizer tool (<https://automeris.io/WebPlotDigitizer/>).

Model development

Ocular PK models were built under R, Version 4.2.2. The models were developed in the following order: The rabbit model was developed and then translated to monkey and, finally, to human. Concentration-time profiles were simulated with the rxode2 package, whereby simulations were performed with a two-fold deviation range, to account for the general high variability of samples from ocular studies (Abduljalil et al., 2014; Yuan et al., 2022). The model was validated by comparison of the predicted profiles to the experimental observations.

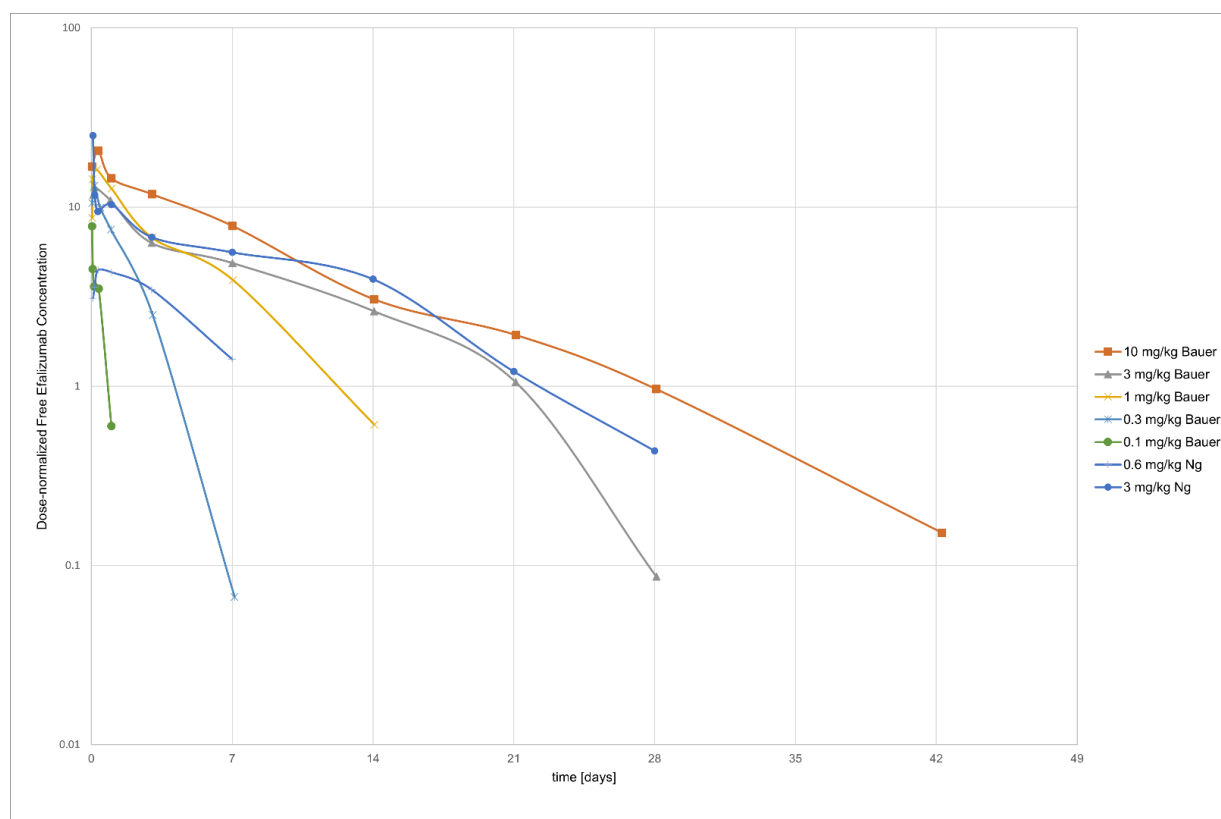
The model structure was earlier described in Chapter 3 and 4. It incorporates the basics of the Hutton-Smith et al. (Hutton-Smith et al., 2017) model, including the injected eye vitreous, aqueous and retina, extended by a plasma compartment and a peripheral compartment.

Parameters for ocular compartment volumes and plasma volumes were obtained from literature (Gaudreault et al., 2005; Feher, 2012; Missel, 2012; Gadkar et al., 2015) and retinal surface area and retinal volume were calculated as described in Hutton-Smith et al. (Hutton-Smith et al., 2017). The vitreous was considered as a sphere, partially covered by the retina, which depicts a circular cross section through the vitreous.

Appendix

Supplementary Material Chapter 2

Supplementary Figures



Supplementary Figure 1. Dose-normalized plot of the observed plasma efalizumab concentrations following intravenous administration in human. Data were obtained from literature (Bauer et al., 1999; Ng et al., 2005). Data from Ng et al. were not considered for model development as the concentration-time profile for the 0.6 mg/kg dose does not align well with the other observed data (early measurements are not lying between the observations of 0.3 mg/kg dose and 1 mg/kg dose).

Supplementary Tables

Supplementary Table 1. Local sensitivity analysis of the human efalizumab PBPK model performed in OSP suite. CD11a(ref) CD11a reference concentration, K_D equilibrium dissociation rate constant of the drug-target-complex, $K_{D,FcRn}$ equilibrium dissociation rate constant of the drug-FcRn-complex, $k_{int,C}$ internalization rate of the drug-target-complex, $k_{int,T}$ internalization rate of the target, k_{off} dissociation rate constant of the drug-target-complex.

Parameter	AUC extrapolated to infinity, dose 0.1 mg/kg	AUC extrapolated to infinity, dose 0.3 mg/kg	AUC extrapolated to infinity, dose 1 mg/kg	AUC extrapolated to infinity, dose 3 mg/kg	AUC extrapolated to infinity, dose 10 mg/kg
$K_{D,FcRn}$	- 0.04	- 0.15	- 0.5	- 0.91	- 1.25
CD11a(ref)	- 5.55	- 2.15	- 1.23	- 0.3	- 0.12
$k_{int,T}$	- 0.13	- 0.34	- 0.5	- 0.22	- 0.09
$k_{int,C}$	- 0.82	- 0.2	- 0.0095	- 0.0075	- 0.0019
k_{off}	- 0.11	- 0.03	- 0.011	- 0.0011	- 0.00026
K_D	0.46	0.07	0.024	0.0026	0.00062

Supplementary Material Chapter 3

Model code

```
## template ocular PK model for simulation ivt administered compounds in rabbits  
with eight compartments: vitreous, aqueous, retina (injected and fellow eye); plasma;  
peripheral;
```

```
## case exapmle: bevacizumab
```

```
## adjusted model assumes an additional exchange pathway between plasma and  
aqueous, as well as an adjusted retinal volume via f_ret
```

```
## units: days, pmol, ml, cm
```

```
## parameters: ----
```

```
#Aqueous humor clearance, Claq;
```

```
#plasma elimination rate constant, ke_plas;
```

```
#transfer rate between plasma and aqueous chamber (only in final model), kplas_aq;
```

```
#diffusion rate constant from vitreous to aqueous chamber, kvit_aq;
```

```
#bevacizumab permeability of the inner limiting membrane, pILM_beve;
```

```
#bevacizumab permeability of the retinal pigment epithelium, pRPE_beve;
```

```
#distribution rate between plasma and peripheral compartment, Q;
```

```
#retinal surface area, Sret; volume of aqueous, Vaq;
```

```
#volume of peripheral compartment, Vperi;
```

```
#volume of plasma, Vplas;
```

```
#volume of retina, Vret;
```

```
#volume of vitreous, Vvit;
```

```
# load libraries----
```

```
library(dplyr)
```

```
library(ggplot2)
```

```
require(readxl)
```

```
library(rxode2)
```

```
# load observed data file with the name "template_ivt_PK_model_data.xlsx"
# containing different sheets for Bakri et al., Sinapis et al., internal;----

data_bakri <- read_excel("template_ivt_PK_model_data.xlsx",sheet = "BAKRI") #
units pmol/ml

data_internal <- read_excel("template_ivt_PK_model_data.xlsx",sheet =
"INTERNAL") # units pmol/ml

## rxode----

# accessible retinal volume fraction:

f_ret=0.41

theta <- c(
  # fixed parameters:
  Cl_aq = 4.32 # ml/day; Missel et al.
  ,v_aq = 0.325 #ml; Missel et al.
  ,v_ret =0.044*f_ret #ml; Missel et al.
  ,v_vit =1.52 #ml; Missel et al.
  ,S_ret =4.35 # cm2; Hutton-Smith et al.
  ,v_plas=80 # ml; Gadkar et al.

  # compound-specific parameters (adjust for respective compound!):
  # the following values were estimated in Phoenix
  ,kvit_aq_beve=0.147 #1/day;
  ,v_peri=104 # ml;
  ,Q_dist=1030# ml/day;
  ,ke_plas=0.339 # 1/day;
  ,pILM_beve =35.9 # cm/day;
```

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,pRPE_beava=0.00051 # cm/day; base model: 0.0159 Hutton-Smith et al.; adjusted model: 0.00051

,kplas_aq=0.024 #ml/day; # set to 0 if no fellow eye aqueous data available

)

beva.ode <- RxODE({

 d/dt(beva_vit) <- (S_ret/v_vit)*pILM_beava*beva_ret -
(S_ret/v_vit)*pILM_beava*beva_vit - kvit_aq_beava*beva_vit

 d/dt(beva_aq) <- (v_vit/v_aq)*kvit_aq_beava*beva_vit - (Cl_aq/v_aq)*beva_aq +
kplas_aq*beva_plas/v_aq - kplas_aq*beva_aq/v_aq

 d/dt(beva_ret) <- (S_ret/v_ret)*pILM_beava*beva_vit -
(S_ret/v_ret)*pILM_beava*beva_ret - (S_ret/v_ret)*pRPE_beava*beva_ret +
(S_ret/v_ret)*pRPE_beava *beva_plas

 d/dt(beva_plas) <- - Q_dist*beva_plas/v_plas + Q_dist*beva_peri/v_plas +
Cl_aq*beva_aq/v_plas - ke_plas*beva_plas + Cl_aq*beva_faq/v_plas +
(S_ret/v_plas)*pRPE_beava*beva_ret + (S_ret/v_plas)*pRPE_beava*beva_fret -
2*(S_ret/v_plas)*pRPE_beava *beva_plas - 2*kplas_aq*beva_plas/v_plas +
kplas_aq*beva_aq/v_plas + kplas_aq*beva_faq/v_plas

 d/dt(beva_peri) <- Q_dist*beva_plas/v_peri - Q_dist*beva_peri/v_peri

 d/dt(beva_fvit) <- (S_ret/v_vit)*pILM_beava*beva_fret -
(S_ret/v_vit)*pILM_beava*beva_fvit - kvit_aq_beava*beva_fvit

 d/dt(beva_faq) <- (v_vit/v_aq)*kvit_aq_beava*beva_fvit - (Cl_aq/v_aq)*beva_faq +
kplas_aq*beva_plas/v_aq - kplas_aq*beva_faq/v_aq

 d/dt(beva_fret) <- (S_ret/v_ret)*pILM_beava*beva_fvit -
(S_ret/v_ret)*pILM_beava*beva_fret - (S_ret/v_ret)*pRPE_beava*beva_fret +
(S_ret/v_ret)*pRPE_beava *beva_plas

})

#dosing

v_vit <- 1.52 # ml

Appendix

```
dose_125 <-8389/v_vit
```

```
## administration and run simulations
```

```
# unilateral injection of 1.25 mg:
```

```
evt_tbl_125mg_bakri <- et() %>%
```

```
  et(dose=dose_125, addl=0, ii=0, dur=0, cmt="beva_vit") %>%
```

```
  et(0, 70, by=0.1) # sample concentration from 0 to 70 days in 0.1 days steps
```

```
run_1p25_unilat <-rxSolve( beva.ode, theta, evt_tbl_125mg_bakri)
```

```
# bilateral injection of 2x 1.25 mg:
```

```
evt_tbl_125mg_2eyes <- et() %>%
```

```
  et(dose=dose_125, addl=0, ii=0, dur=0, cmt="beva_vit") %>%
```

```
  et(dose=dose_125, addl=0, ii=0, dur=0, cmt="beva_fvit") %>%
```

```
  et(0, 70, by=0.1)
```

```
run_1p25_bilat <- rxSolve( beva.ode, theta, evt_tbl_125mg_2eyes)
```

```
## plot simulated and observed data
```

```
plot_theme <- theme(panel.background = element_rect(fill="white"),
```

```
  panel.grid.major.x = element_line(color="grey90"),
```

```
  panel.grid.major.y = element_line(color="grey90"),
```

```
  panel.grid.minor.x = element_blank(),
```

```
  panel.grid.minor.y = element_blank(),
```

```
  axis.title = element_text(color = "black", size = 10),
```

```
  axis.text.x = element_text(color = "black", size = 8),
```

```
  axis.text.y = element_text(color = "black", size = 8),
```

```
axis.line      = element_line(color="black", size = 1),  
axis.text.x.top = element_blank(),  
axis.ticks.x.top = element_blank(),  
axis.line.x.top = element_blank(),  
panel.border = element_rect(colour = "black", fill=NA, size=1))
```

```
# plot injected eye aqueous
```

```
aq_inj<-ggplot() +
```

```
  geom_line(data=run_1p25_bilat, aes(x=time, y=beva_aq), cex=1,color="violet") + #  
  Plot sim aq concentrations
```

```
  geom_line(data=run_1p25_unilat, aes(x=time, y=beva_aq),  
    cex=1,color="darkgreen") +
```

```
  geom_point(data=filter(data_bakri,DVID=="beva_aq"), aes(x=TIME,  
    y=DV,color="Bakri"), cex=1.5) + # Bakri
```

```
  geom_point(shape=15,data=filter(data_internal,DVID=="beva_aq"), aes(x=TIME,  
    y=DV,color="internal"), cex=1.5) +
```

```
  scale_colour_manual("", values = c("internal"="violet","Bakri"="darkgreen" )) +
```

```
  labs(title="Aqueous (injected eye)", y=paste0("Concentration (nM)"), x= "Time  
(days)") +
```

```
  scale_x_continuous(breaks=c(seq(0, 72, 12), seq(72, 672, 48)),limits = c(0, 40)) +
```

```
  scale_y_log10(limits = c(0.1, 1000)) +
```

```
  annotation_logticks(sides = "l") +
```

```
  plot_theme
```

```
# plot injected eye vitreous
```

```
vit_inj<-ggplot() +
```

```
  geom_line(data=run_1p25_unilat, aes(x=time, y=beva_vit),  
    cex=1,color="darkgreen") +
```

```
  geom_line(data=run_1p25_bilat, aes(x=time, y=beva_vit), cex=1,color="violet") +
```

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```
geom_point(data=filter(data_bakri,DVID=="beva_vit"), aes(x=TIME,
y=DV,color="Bakri"), cex=1.5) + # Bakri

geom_point(shape=15,data=filter(data_internal,DVID=="beva_vit"), aes(x=TIME,
y=DV,color="internal"), cex=1.5) +

scale_colour_manual("", values = c("internal"="violet","Bakri"="darkgreen" )) +

labs(title="Vitreous (injected eye)", y=paste0("Concentration (nM)"), x= "Time
(days)") +

scale_x_continuous(breaks=c(seq(0, 72, 12), seq(72, 672, 48)),limits = c(0, 40)) +

scale_y_log10(limits = c(10, 10000)) +

annotation_logticks(sides = "l") +

plot_theme

# plot injected eye retina;

# Note that observed retinal data reflect the concentrations related to the whole
retinal volume, as measurements were taken from the complete retina sample
including the inaccessible fraction;

ret_inj<-ggplot() +

geom_line(data=run_1p25_bilat, aes(x=time, y=beva_ret*f_ret),
cex=1,color="violet") + # Plot sim aq concentrations

geom_point(shape=15,data=filter(data_internal,DVID=="beva_ret"), aes(x=TIME,
y=DV,color="internal"), cex=1.5) +

scale_colour_manual("", values = c("internal"="violet","Bakri"="darkgreen" )) +

labs(title="Retina (injected eye)", y=paste0("Concentration (nM)"), x= "Time (days)")
+

scale_x_continuous(breaks=c(seq(0, 72, 12), seq(72, 672, 48)),limits = c(0, 40)) +

scale_y_log10(limits = c(10, 10000)) +

annotation_logticks(sides = "l") +

plot_theme

# plot plasma

plas_plot<-ggplot() +
```

Appendix

```
geom_line(data=run_1p25_unilat, aes(x=time, y=beva_plas),
cex=1,color="darkgreen") + # Plot sim aq concentrations

geom_line(data=run_1p25_bilat, aes(x=time, y=beva_plas), cex=1,color="violet") +

geom_point(shape=15,data=filter(data_bakri,DVID=="beva_plas"), aes(x=TIME,
y=DV,color="Bakri"), cex=1.5) + # Bakri

geom_point(data=filter(data_internal,DVID=="beva_plas"), aes(x=TIME,
y=DV,color="internal"), cex=1.5) +

scale_colour_manual("", values = c("internal"="violet","Bakri"="darkgreen" )) +

labs(title="Plasma", y=paste0("Concentration (nM)"), x= "Time (days)") +

scale_x_continuous(breaks=c(seq(0, 72, 12), seq(72, 672, 48)),limits = c(0, 60)) +

scale_y_log10(limits = c(0.001, 100)) +

annotation_logticks(sides = "l") +

plot_theme
```

plot fellow eye aqueous

```
fel_plot_beava_aq<-ggplot() +

geom_line(data=run_1p25_unilat, aes(x=time, y=beva_faq),
cex=1,color="darkgreen") + # Plot sim aq concentrations

geom_point(data=filter(data_bakri,DVID=="beva_faq"), aes(x=TIME,
y=DV,color="Bakri"), cex=1.5) + # Bakri

scale_colour_manual("", values = c("Bakri"="darkgreen" )) +

labs(title="Aqueous (fellow eye)", y=paste0("Concentration (nM)"), x= "Time
(days)") +

scale_x_continuous(breaks=c(seq(0, 72, 12), seq(72, 672, 48)),limits = c(0, 40)) +

scale_y_log10(limits = c(0.0001, 1)) +

annotation_logticks(sides = "l") +

plot_theme
```

plot fellow eye vitreous

```
fel_plot_beava_vit<-ggplot() +
```

Appendix

```
geom_line(data=run_1p25_unilat, aes(x=time, y=beva_fvit),
cex=1,color="darkgreen") + # Plot sim aq concentrations

geom_point(data=filter(data_bakri,DVID=="beva_fvit"), aes(x=TIME,
y=DV,color="Bakri"), cex=1.5) + # Bakri

scale_colour_manual("", values = c("Bakri"="darkgreen" )) +

labs(title="Vitreous (fellow eye)", y=paste0("Concentration (nM)"), x= "Time (days)")
+

scale_x_continuous(breaks=c(seq(0, 72, 12), seq(72, 672, 48)),limits = c(0, 40)) +

scale_y_log10(limits = c(0.0001, 10)) +

annotation_logticks(sides = "l") +

plot_theme

# side by side plot

library(cowplot)

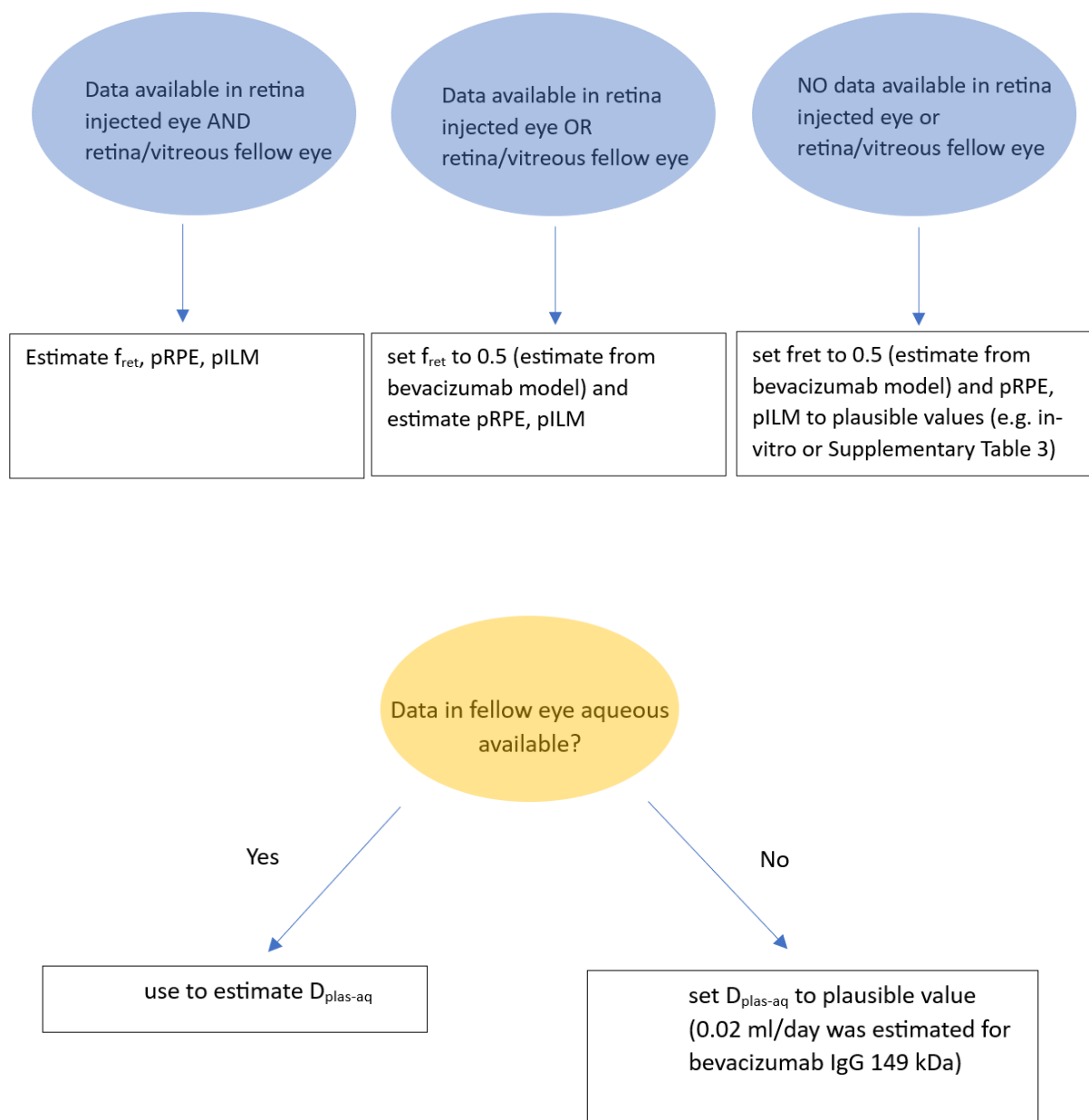
full_plot<-plot_grid(ret_inj,vit_inj,
aq_inj,plas_plot,fel_plot_beve_vit,fel_plot_beve_aq,labels = "AUTO")

full_plot
```

User guide: Ocular and systemic PK model with or without fellow eye

1. Fix species-dependent anatomical/physiological parameters (Supplementary Table 2)
2. Estimate the parameters k_{vit-aq} , Q , V_{peri} , $k_{e,plas}$ from data
3. Fix or estimate f_{ret} , $pRPE$, $pILM$, $D_{plas-aq}$ according to scenario 1 or 2 (see below)

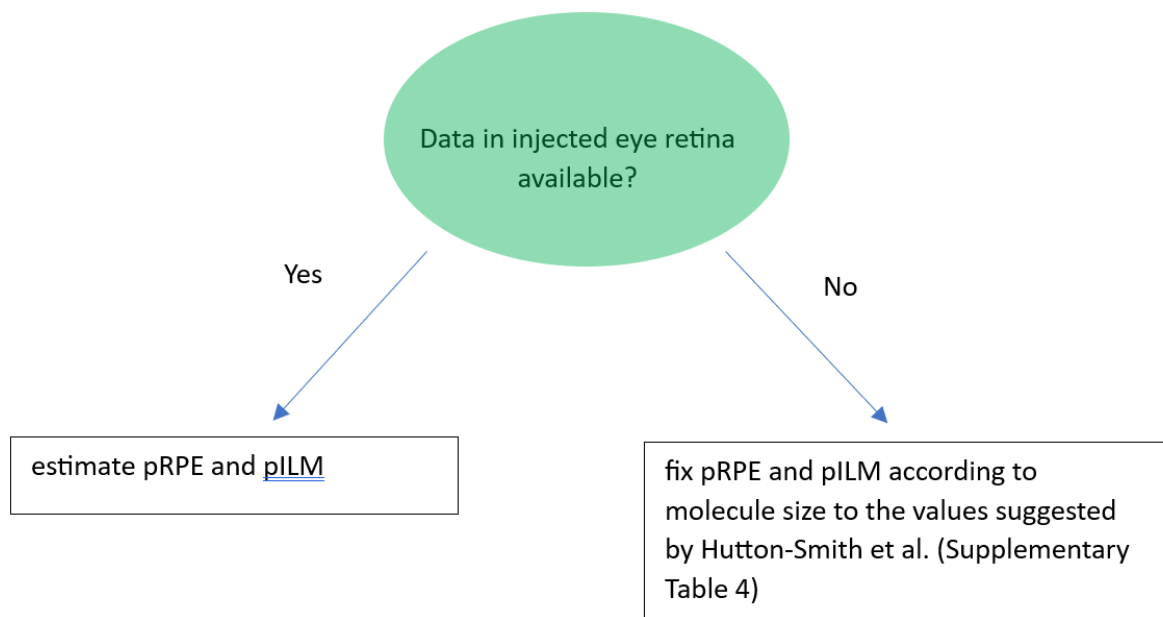
Scenario 1: Fellow eye data are available OR you want to make predictions for the fellow eye (“refined model”)



Scenario 2: No fellow eye data are available AND no predictions for the fellow eye necessary ("base model")

Simplify the model (the model will assume that the drug enters the whole retinal volume, even cells; however, this simplification does not influence the injected eye retina, only the fellow eye):

set $f_{\text{ret}} = 1$; $D_{\text{plas-aq}} = 0$



Supplementary Tables

Supplementary Table 2. Species-dependent anatomical/physiological parameters.

Parameter	Rabbit model	Monkey model	Human model	Source	Description
V_{vit} [ml]	1.52	2.17	4.5	Missel et al., 2012	Volume of vitreous
V_{aq} [ml]	0.325	0.134	0.16	Rabbit, Monkey: Missel et al., 2012 Human: Hutton-Smith et al., 2018	Volume of aqueous chamber
V_{ret} [ml]	0.044	0.104	0.22	Missel et al., 2012	Volume of retina
S_{ret} [cm ²]	4.35	5.09	9.71	Rabbit: Hutton-Smith et al., 2017 Human: Hutton-Smith et al., 2018 Monkey: calculated according to Hutton-Smith et al., 2017	Retinal surface area
Cl_{aq} [ml • day ⁻¹]	4.32	2.45	(3.6)	Rabbit, Monkey: Missel et al., 2012 Human patients might have altered clearance due to disease → estimate	Aqueous humor clearance
V_{plas} [ml]	80	126	2900	Rabbit: Gadkar et al., 2015	Volume of plasma compartment

Appendix

				Monkey: Gaudreault et al., 2005 Human: Feher et al., 2012	
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Supplementary Table 3. Plausible values for pILM and pRPE in the model with adjusted retinal volume if estimation is not possible.

	~150 kDa drug	~50 kDa drug
pILM [$\text{cm} \cdot \text{s}^{-1}$]	1.7e-7 (Hutton-Smith et al., 2017)	1.88e-7 (Hutton-Smith et al., 2017)
pRPE [$\text{cm} \cdot \text{s}^{-1}$]	5.9e-9 (estimated from bevacizumab fellow eye data)	1.8e-8 (Terasaki et al., 2015)

Supplementary Table 4. Plausible values for pILM and pRPE (Hutton-Smith et al., 2017) in the model without adjustment of retinal volume if estimation is not possible.

	~150 kDa drug	~50 kDa drug
pILM [cm • s⁻¹]	1.7e-7	1.88e-7
pRPE [cm • s⁻¹]	1.84e-7	2.6e-7

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