FACULTY OF PHARMACEUTICAL SCIENCES

LITERATURE SEARCH CONCERNING THE PHARMACOKINETICS OF DRUGS IN THE EYE

Mica Angela Gomeri

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ABSTRACT

Introduction: Ocular diseases affect millions of individuals worldwide. As the world's population ages, the incidence of several vision-impairing, age-related ocular diseases are on the rise. To develop effective and safe drugs for these ocular diseases, the pharmacokinetics (PK) of such drugs need to be observed and understood before proceeding to clinical trials. The eye, however, is a complex, well-protected organ with several barriers. As a result, ocular drug delivery faces several challenges. Because of the invasive sampling of ocular fluids, ocular PK studies are not often conducted in human. Researchers therefore turn to animal eyes for research, mostly those of rabbits, to extrapolate to humans. Physiologically-Based Pharmacokinetic (PBPK) modelling is a computational tool that can help in ocular drug development by predicting the PK of a compound in different situations. These situations include different species, dosage forms, dosing scheme, etc. Validation of these models with preclinical or clinical data are then necessary to ensure their reliability in predicting *in vivo* PK, thereby aiding in rational decision making in drug development.

Objective: 1) To provide a comprehensive overview of current knowledge on ocular PK after topical and intravitreal administration. 2) To conduct a few PBPK modelling experiments to understand PBPK modelling in the context of ocular PK and to integrate the knowledge gained from the literature search.

Methods: A literature search was conducted to find the most recent literature on ocular PK. The current knowledge on ocular PK was then provided. For the PBPK modelling experiments, another literature search was conducted to find suitable rabbit and human ocular PK studies. Afterwards, PBPK modelling was performed in GastroPlus® using the integrated ocular model, namely the OCAT™ model.

Results: A structured overview was provided for both the anterior segment (topical) PK and posterior segment (intravitreal) PK of the eye. The ocular model of intravitreal triamcinolone acetonide (TA) showed good predictions in rabbit but not in human. The validated models for topical levofloxacin and gatifloxacin unable to capture the observed data of the respective oral studies.

Conclusion: The PK of topically administered drugs is more extensively documented and described than that of intravitreally administered drugs in the available literature. Furthermore, there are several topics that still need clarification and validation *in vivo*. Although the modelling of TA did not capture the observed human values, the model does give us predictions on how the ocular PK could look like in humans in different matrices. Due to the lack of human PK data, validation of this model is not possible. The second experiment shows us that although an ocular model is validated for topical solutions, it may not be able to capture the PK of the same drug after oral administration.

SAMENVATTING

Introductie: Oogziekten treffen miljoenen mensen wereldwijd. Door de verouderende bevolking stijgt de incidentie van verschillende oogziekten die blindheid kunnen veroorzaken. Om veilige, doeltreffende geneesmiddelen te ontwikkelen tegen deze ziekten, is kennis van de farmacokinetiek (PK) van oculaire geneesmiddelen cruciaal vooraleer men verdergaat naar klinische studies. Het oog is een complex, goed beschermd orgaan met verschillende barrières. De toediening van oculaire geneesmiddelen is hierdoor sterk gehinderd. Door de invasieve bemonstering van het oogstalen, zijn PK studies in het oog nauwelijks uitgevoerd bij mensen. Onderzoekers zijn daarom genoodzaakt om proefdierogen, meestal die van konijnen, te gebruiken voor geneesmiddelenontwikkeling. Op die manier wordt er geëxtrapoleerd van konijn naar mens. Fysiologisch gebaseerd farmacokinetische (PBPK) modellering is een computationeel hulpmiddel dat kan helpen om de PK van geneesmiddelen in verschillende situaties te voorspellen. Deze situaties omvatten verschillende diersoorten, doseringsvormen, doseringsschema's, etc.

Objectieven: 1) Om door middel van literatuuronderzoek een uitgebreid overzicht te geven van de huidige kennis over oculaire PK na topicale en intravitreale toediening. 2) Om een aantal proeven uit te voeren met PBPK modellering in het oog en om de kennis, verkregen door het literatuuronderzoek, te integreren.

Methoden: Literatuuronderzoek werd uitgevoerd om de meest recente wetenschappelijke literatuur over oculaire PK te vinden. De huidige kennis over dit thema werd weergegeven. Voor de PBPK modellering proeven werd een ander literatuuronderzoek uitgevoerd om geschikte data bij proefkonijnen en mens te vinden. Daarna werd PBPK modellering uitgevoerd in GastroPlus® met het geïntegreerde oculair model, namelijk het OCAT™ model.

Resultaten: Een gestructureerd overzicht werd weergegeven voor de PK van het voorste (topicaal) en achterste (intravitreaal) segment van het oog. Het oculaire model van intravitreaal triamcinolone acetonide (TA) gaf goede predicties bij konijn, maar niet bij de mens. De gevalideerde modellen voor topicale levofloxacine en gatifloxacine waren niet in staat om de geobserveerde waarden in de orale studies te voorspellen.

Conclusie: De PK van topicale geneesmiddelen is uitgebreider gedocumenteerd en beschreven in de huidige literatuur dan de PK van intravitreale geneesmiddelen. Bovendien zijn er nog verschillende aspecten van oculaire PK die verduidelijking en *in vivo* validatie vergen. Hoewel het model van TA niet in staat was om de geobserveerde waarden bij de mens te capteren, geeft het model een idee van hoe de oculaire PK bij de mens zou zijn in verschillende biologische matrices. Door het gebrek aan PK data bij de mens, is validatie van dit model niet mogelijk. Het tweede experiment toont aan dat, hoewel een oculair model gevalideerd is voor topicale oplossingen, het model mogelijks niet in staat is om de PK van hetzelfde geneesmiddel na orale toediening te voorspellen.

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LIST OF USED ABBREVIATIONS

ADME	Absorption, distribution, metabolism, excretion
AH	Aqueous humour
AUC	Area under the curve, drug exposure
BAB	Blood-aqueous barrier
BCRP	Breast cancer resistance protein
BRB	Blood-retinal barrier
CNV	Choroidal neovascularization
ICB	Iris-ciliary body
ILM	Inner limiting membrane
Log D	Partition coefficient (at experimental pH), lipophilicity
Log P	Partition coefficient (at neutral pH), lipophilicity
mAbs	Monoclonal antibodies
MRP	Multidrug resistance protein
OATP	Organic anion transporting polypeptides
OCAT™	Ocular Compartment Absorption & Transit model
Papp	Apparent permeability
PBPK	Physiologically based pharmacokinetic modelling
P-gp	P-glycoprotein
PK	Pharmacokinetics
PSA	Polar surface area
QSPR	Quantitative structure-property relationship modelling
RPE	Retinal pigment epithelium

$t_{1/2}$	Half-life
TA	Triamcinolone acetonide
V_d	Volume of distribution
VEGF	Vascular endothelial growth factor
VH	Vitreous humour

1 INTRODUCTION

1.1 GENERAL

Given its widespread occurrence, it is nearly inevitable for most individuals to encounter at least one eye condition throughout their lives, albeit a non-vision impairing condition such as e.g. dry eye. According to the World report on vision (2019) of the World Health Organization, over 2.2 billion people worldwide have one or more visual disorders that lead to vision impairment. At least 1 billion people in this group have vision impairment that could have been prevented (untreated cataract, glaucoma, age-related macular degeneration, diabetic retinopathy, etc.) (1). The incidence of these diseases is ever growing, due to the increase of the world's aging population (2). As a result, there is a need for the development of innovative drugs to address these ocular diseases. During the drug development process, the specific pharmacokinetics (PK) of the drug are studied early on. PK is the study that explores and describes the fate of drugs in the body, essentially, how the body processes xenobiotic substances, which are in the context of this thesis, medicinal drugs. Systemic PK is well-known and described, forming the foundation of the overall principles of the study. However, due to the intricate anatomy and various physiological processes in the eye, ocular PK in itself is a different system altogether. Understanding the PK of ocular drugs is crucial to optimize drug development and to ensure the safety and effectiveness of the drug for patients. Conversely, obtaining samples from the eye is highly invasive and requires careful consideration. In reality, this often results in sparse sampling of ocular fluids or tissues. Researchers tend to resort to animal models to study ocular PK, aiming to extrapolate their findings to humans. The use of animal models has become a standard practice to improve our understanding in ocular PK and ocular drug development. Nonetheless, according to Directive 2010/63/EU of the European Commission, it is required that the number of animals used for scientific experiments be reduced as much as possible, following the 3R principle (Reduction, Refinement, and Replacement of animal testing) (3,4).

1.2 THE EYE

To fully understand the PK of the eye, it is important to start at the anatomy and physiological structure of the eye, along with the physiological barriers that drugs need to overcome.

1.2.1 Anatomy and physiology

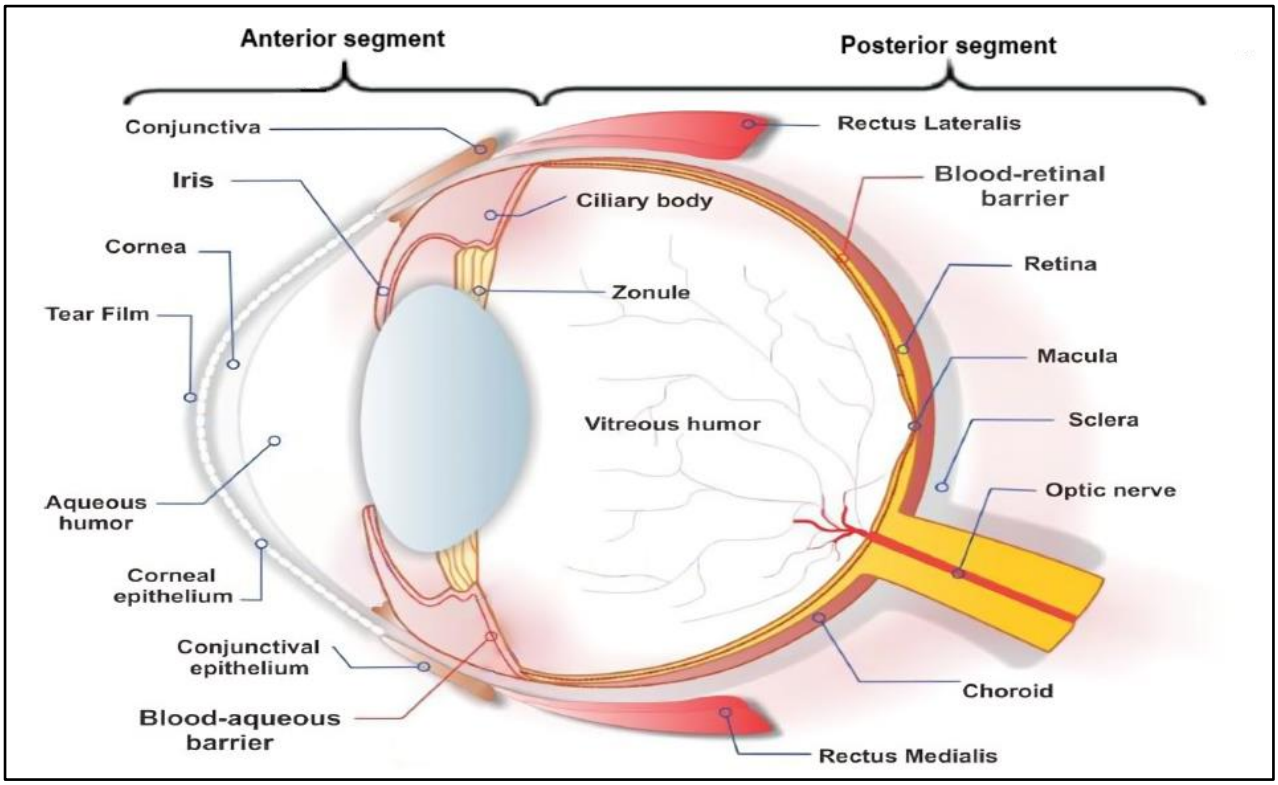

Figure 1.1: Schematic illustration of the anatomy of the eye. Adapted from Reference (5).

The global structure of the eye can be split into two segments: the anterior segment and the posterior segment. The anterior segment is exposed to the outside world and consists of the cornea, anterior chamber, aqueous humour (AH), lens, conjunctiva and iris-ciliary body (ICB). The posterior segment is larger and consists of the vitreous cavity (containing the vitreous humour (VH)), retina, choroid and sclera (6–8).

Each of these anatomical structures have their distinctive functions. The brain processes visual stimuli through light, which is conducted through the cornea and crosses the anterior segment filled with AH. The light then crosses the lens and passes through the VH, finally impacting the retina which then transmits the signal to the brain via the optic nerve. The choroid and ICB form the vascular tissue of the eye. The main blood supply for the whole eye, however, is provided by the ophthalmic artery and the choroidal plexus. This way, the structures of the eye are able to receive nutrients or drugs that are present in the systemic circulation (7,9–12).

Structures surrounding the eye globe are also of importance, as they could impact the amount of drug in the eye. These structures are the eyelids, lacrimal system, and the tear film. The eyelids allow blinking to spread tears, produced by the lacrimal system, over the eye. A part of these tears could then drain into the nasolacrimal duct. After

drainage, the tears get swallowed and reach the stomach (13). The tear film is comprised of multiple layers, each produced by different glands and cells. From anterior to posterior, the tear film is comprised of the superficial lipid layer, the aqueous layer and the internal layer. The superficial lipid layer stabilizes the tear film and reduces evaporation of underlying layers. The aqueous layer in the middle contains proteins and water-soluble salts. Lastly, the internal layer is primarily composed of mucin and adheres to the microvilli of corneal epithelial cells, facilitating retention and even distribution of the aqueous layer across the hydrophobic corneal surface (9–12).

1.2.2 Ocular barriers

Anterior segment barriers

Depending on the administration route, the drug must overcome various barriers to reach the site of action. The anterior segment has several barriers that can be divided into physiological barriers and anatomical barriers. Physiological barriers in the anterior segment consist of tear turnover, nasolacrimal drainage and blinking. Tear turnover encompasses tear production and drainage of tears in the eye. Nasolacrimal drainage is the process where tears, whether or not containing drugs, drain into the nasolacrimal duct where they could flow to the gastro-intestinal tract and get absorbed systemically (14). The anatomical barriers can be further divided into static and dynamic barriers. The static barriers include the corneal epithelium, the stroma and the blood-aqueous barrier (BAB). The corneal epithelium is the lipophilic, outmost layer of the cornea and contains tight junctions, causing difficulty for topically administered drugs to permeate through paracellularly (14,15). This is because the epithelium itself is comprised of several layers, with the outermost layers consisting of squamous superficial cells. These cells possess tight intercellular junctions, limiting the absorption of small molecule hydrophilic drugs (16,17). Proteins are unable to pass through this barrier at measurable rates, while hydrophilic small molecular drugs permeate slowly. Conversely, small, lipid-soluble compounds can permeate faster, due to the partitioning into the epithelial cell membranes consisting mainly of lipids (18,19). Subsequently, the stroma, one of the several corneal layers, is hydrophilic in nature and constitutes the majority of the corneal thickness (14,20).

Lastly, the anterior BAB consists of various structures, including the non-pigmented epithelial cells of the ciliary body, intercellular junctions and blood vessels of the iris. The dynamic barriers consist of conjunctival blood flow, lymph flow and tear drainage (14,15). These three processes are involved in the clearance of xenobiotics and metabolites present in the eye (14,17). Subsequently, the posterior segment consists of static and dynamic barriers, with the former

including the conjunctiva, Brunch's membrane-choroid, and the retinal pigment epithelium (RPE). The latter comprises blood and lymphatic vessels, similar to the anterior segment (5,21,22).

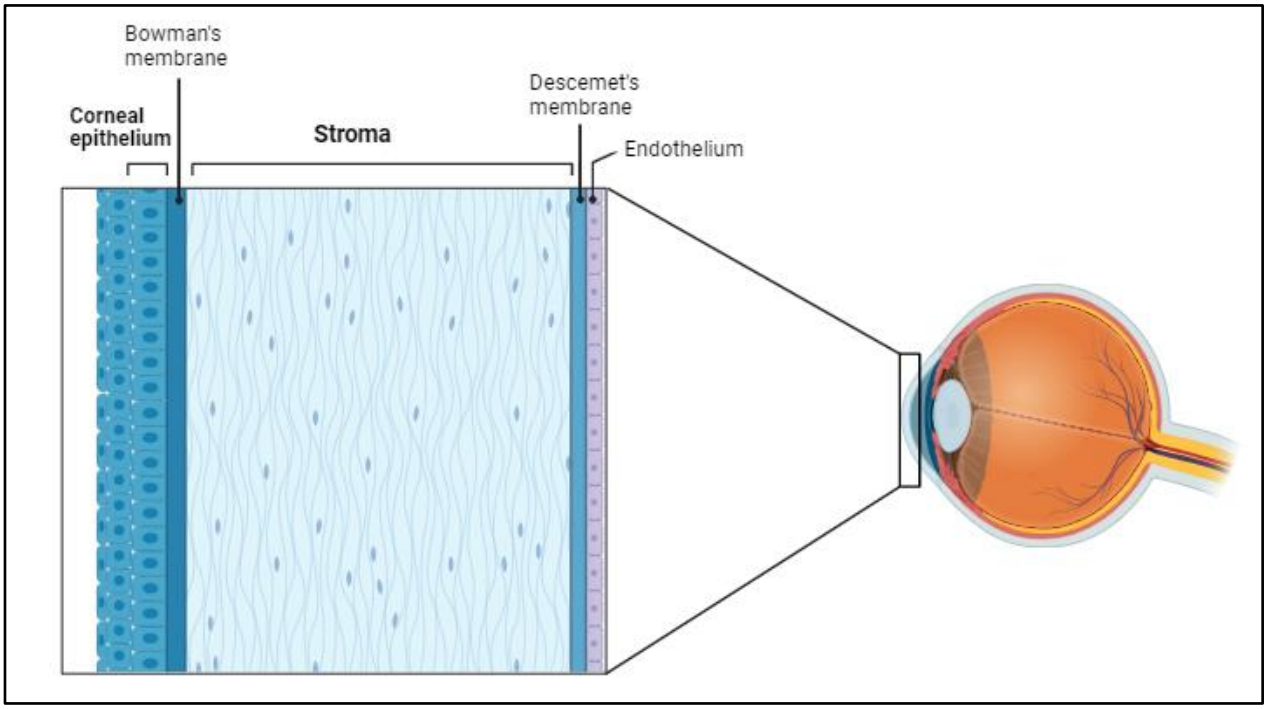

Figure 1.2: Schematic illustration of the corneal layers. Created with BioRender.com (23).

The blood-ocular barriers protect the eye from xenobiotics that could be present in the systemic circulation. These barriers consist of two components: the anterior BAB and the posterior blood-retinal barrier (BRB), each characterized by epithelial and endothelial tight junctions that limit drug transport (17,24). The BAB restricts the access of drugs from the systemic circulation into the AH through the capillaries in the ICB (5,15,16,21,22). Although the BAB forms an important barrier for xenobiotics present in the systemic circulation, its function is not absolute. It has been shown that systemically administered horse radish peroxidase was able to permeate into the AH, not through the iris blood vessels, but through the fenestrated capillaries of the ciliary body (25).

Additionally, mucin forms another ocular barrier. Mucin consists of various hydrophilic, anionic mucin proteins that are present on the surface of the cornea (through the tear film) and conjunctiva, constituting a protective role of the surface of the eye. Mucin is seen as a permeation barrier, meaning that it restricts the absorption of topical drugs

through the cornea, however, there is no consensus on its influence on the drug's bioavailability. Permeability through this barrier is primarily determined by molecular weight, with the ionic charge and hydrophilicity playing secondary roles (26). The diffusion of molecules with a large molecular weight (i.e. molecules that are larger than the mesh size of the gel network of the different mucins) may be hindered, due to the slower rate of permeation (14,26). Because the mucus mesh size is generally less than 1 mm, it acts as a barrier to particles in the micrometre range (27). Furthermore, even synthetic nanoparticles that are smaller than 500 nm are susceptible to polyvalent interactions, such as electrostatic interactions, with mucins and will therefore be trapped (27–29). The anionic mucin attracts cationic compounds and repulses negatively charged compounds (30). Moreover, lipophilic compounds have been shown to exhibit low permeability in mucin, due to their binding to mucin itself (31). It can therefore be concluded that permeability through ocular mucins is defined by diffusion through the mesh network and interactions with mucus (26).

Additionally, efflux pumps in the eye, located on the cornea and retina, play a crucial role in drug absorption (32). Examples of such efflux pumps include P-glycoprotein (P-gp), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP). These efflux transporters excrete xenobiotic molecules after they diffuse inside the cell, resulting in reduced toxic exposure on one hand, but also possibly decreased bioavailability on the other (14).

Posterior segment barriers

The posterior compartment barriers consist of the VH, the inner limiting membrane (ILM), the retina, BRB, choroid, and sclera (33). Firstly, the VH itself acts as a barrier, both static and dynamic, to intravitreally administered drugs. It is a static barrier due to the composition of the VH, which mainly consists of collagen fibres and glycosaminoglycans (mostly hyaluronic acid). However, the structure is not significantly restrictive for molecular distribution. Additionally, the dynamic aspect refers to the flow and clearance processes in the vitreous (33).

In addition, the ILM is a nearly impenetrable barrier positioned between the vitreous chamber and the retina (34). The retina is a structure composed of seven layers. Due to the Muller glial cells between the ILM and outer limiting membrane of the retina, molecules are not able to diffuse easily through this barrier (33–35).

Moreover, both layers of the BRB, namely the inner BRB and outer BRB, limit drug delivery from the systemic circulation to the retina (36). The outer BRB, also known as the RPE, is rich in melanin pigment and is surrounded by capillaries in the choroid (35,37,38). Furthermore, active transport of molecules is possible, due to the protein pumps present on the outer BRB and the uptake of molecules into the cell through vesicle invagination (endocytosis) (39).

The inner BRB consists of retinal capillary endothelial cells (7). Both paracellular and transcellular diffusion of molecules through the BRB is possible, although both processes are limited, because fenestrations are absent and transport vehicles are lacking in endothelial cells (33). Additionally, the BRB also restricts the diffusion of molecules from the VH to the retina (33).

Furthermore, the choroid, as mentioned earlier, is highly vascularized and is located between the RPE and the sclera. Similar to the VH, the choroid acts as both a static and a dynamic barrier. The former is due to the structure of the choroid and the latter is due to the high blood flow in the choriocapillaris layer of the choroid. This layer additionally consists of fenestrated capillaries with a pore size of 6-12 nm, permitting the diffusion of macromolecules (40) Lastly, the sclera is the whiteish, outer layer of the eye that provides support to the overall structure and can also serve as a barrier to drugs that target the posterior segment (33).

1.3 ANIMAL MODELS

Due to the complex anatomy, physiology and various barriers of the eye, it has been proven difficult to measure and derive ocular PK parameters. In addition, a general biological matrix to measure drug concentration in the eye is not applicable, due to various reasons concerning the invasiveness of extracting ocular fluid, the type of drug, the administration route, and so on. In the past, several biological matrices have been used, like AH, VH, ocular tissue (conjunctival tissue for example), but also more traditional ones such as plasma, serum, and blood. Generally, human PK is measured through plasma sampling after oral or intravenous administration. Sampling human ocular PK for drugs that work locally however, is not simple (14). Extracting AH or VH, not to mention ocular tissue, presents its own challenges due to its invasive nature. Another problem is that the amount of AH and VH is limited, about 200 µL and 4.5 mL respectively (41). Moreover, extracting these ocular fluid samples through various methods such as paracentesis or vitreous tapping are not risk-free for the patient. Paracentesis of the anterior chamber involves the drainage of the AH through a needle, or alternatively, it can be performed through incision with a knife (42). Similarly, vitreous tapping entails the extraction of VH by inserting a needle into the sclera (43). These methods may additionally damage the surrounding ocular structures and tissues and could result in cataracts, endophthalmitis or retinal detachment (44,45). Therefore, samples of these matrices are exceptionally collected when the patients are to undergo ophthalmic surgery, such as cataract surgery (46). As a result, animal models are routinely used to study PK processes like drug distribution in ocular fluids or tissues, which enables extrapolation to human pharmacokinetics (14). Rabbits and monkeys are the most commonly utilized animals among these animal models (41).

During pre-clinical trials in drug development, researchers rely on animal models to study ocular PK, mostly rabbits (47). This due to the ease of handling, low cost, availability, similarity in eye size to humans and the existence of a large database consisting of information from PK-studies done with rabbits (41). Despite the similar eye size, the rabbit eye does differ in several anatomical aspects. For instance, the rabbit retina is less vascularized, the vitreous cavity is smaller, and the lens is larger compared to human eyes (48). Furthermore, the blinking rate and tear volume production differ from humans as well (17). Although these slight differences in anatomical structure and physiology may lead to differences in PK, the overall influence hereof is not fully clarified. Thus, clinical trials in human are essential to validate data obtained from rabbits (49).

1.4 OPHTHALMIC ADMINISTRATION ROUTES AND DOSAGE FORMS

Due to the complex anatomy of the eye, it is not surprising that there are several types of dosage forms to ensure that the drug reaches the target site in the eye by bypassing the various ocular barriers. Additionally, the PK of an ocular drug is dependent on the dosage form and the route of administration. There are numerous types of ocular dosage forms, each with their advantages and disadvantages. Primarily, there are four main administration routes: topical, periocular, intraocular and systemic (50). An overview will be provided of both traditional ophthalmic drug forms and newer, innovative formulations. For more in-depth information about the more complex ophthalmic dosage forms and their formulation, a detailed review has been provided by Baranowski et al. (51).

1.4.1 Topical

Topical dosage forms are the most universally known, typically used when targeting the surface or anterior segment of the eye. There are, however, studies being done to explore topical administration to target the posterior segment (52). Key advantages include patient-convenience, possibility of self-administration, non-invasiveness, drug localization and cost-effectiveness (50,53). The disadvantages of the topical route include low ocular bioavailability, which is attributed to the short precorneal residence time resulting from tear film turnover and nasolacrimal drainage. Additionally, topical drugs face challenges in penetrating epithelial barriers within the eye (50). Despite these disadvantages, topical dosage forms represent 90% of ocular drug administrations (21).

Topical ophthalmic drugs can take various forms, namely liquid, semisolid or solid. Liquid drug formulations include eye drops, ophthalmic solutions and microemulsions. Eye drops, available in water and oil solutions, emulsions, or suspensions with one or more active substances, serve distinct purposes. Ophthalmic solutions are aqueous solutions without an active substance and are typically used for cleansing and rinsing the ocular surface. Lastly, microemulsions

are formed by mixing two immiscible phases, usually an oil and water phase, with the help of a surfactant. This results in nanodrops that are able to adhere to the cornea and act as a drug reservoir, which helps reduce nasolacrimal drainage (54).

Types of semisolid drug forms consist of in situ gels or eye ointments. In situ gels are dosage forms that can transition between solid to gel-like forms depending on the external ocular environment. Furthermore, eye ointments are usually hydrocarbon-based which melt or soften close to human body temperature. Because of the viscosity of these drug forms, the active ingredient is released slowly, similar to a depot system, and is therefore drained slower from the ocular surface. Due to this, the bioavailability of the ocular drug increases (51).

Solid dosage forms, consist of but are not limited to drug-coated contact lenses, ocular inserts, collagen shields, and minidiscs, which offer diverse options for drug delivery (51). Additionally, advanced topical dosage forms using nano- and microparticles, liposomes, dendrimers, and other innovative delivery systems continue to emerge, aiming to optimize drug delivery (51).

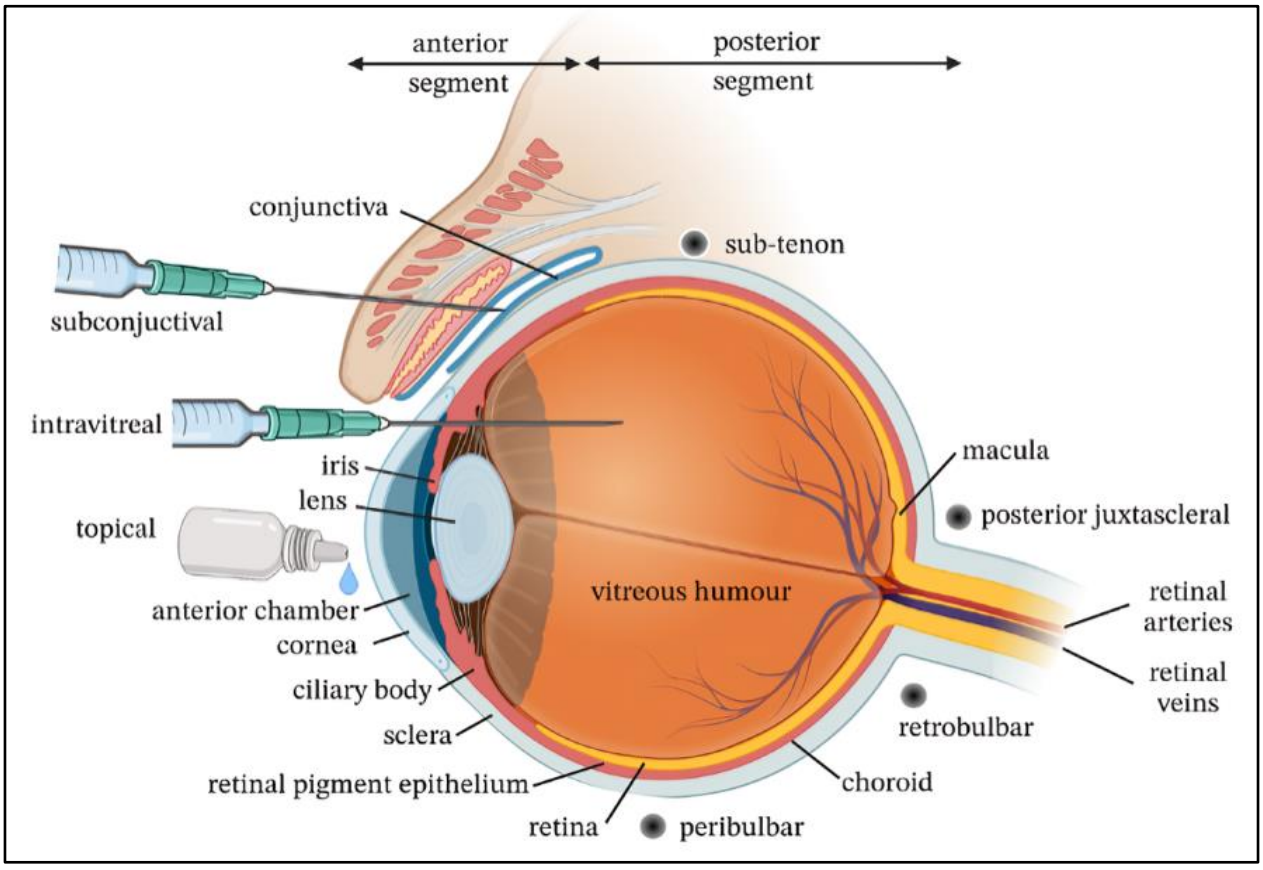

Figure 1.3: Schematic illustration of standard routes of administration. Adapted from Reference (35).

1.4.2 Systemic

Systemic ocular drugs are those that are administered either orally or parenterally (usually intravenously). This route is often used for drugs to reach the posterior segment of the eye; however, it is also possible for systemically administered drugs to reach the AH. This can occur via diffusion from the vitreous into the AH through the ICB, or via permeation through the BAB, as previously discussed (5,25). Systemic administration, like topical administration, is non or minimally invasive, and convenient for patients, therefore making self-administration feasible. However, disadvantages of this administration route are low ocular bioavailability due to the dilution of the drug in blood, and the blood-ocular barriers that the drugs need to permeate to reach the site of action. Due to the low ocular bioavailability, high and frequent doses of the drug need to be administered, which can lead to toxicity and side effects (7). Additionally, systemically administered drugs undergo liver metabolism and kidney clearance, leading to a limited amount of drug that actually reaches the VH. (5,52,55).

1.4.3 Intraocular

Intraocular drug delivery consists of injections in the eye, this way the drug is able to bypass important ocular barriers that hinder drug disposition in the eye when administered topically. Intraocular administration is done to target the posterior segment of the eye. Intravitreal, subretinal, intracameral (injection in the anterior chamber, commonly used to treat glaucoma (56)), intracorneal, and intrascleral administration are all examples of intraocular administration. Fig. 1.3 presents a schematic illustration to visualise where these different injections are localised in the eye. These various depths of injections into the eye allow the drug to overcome several or even all anterior ocular barriers, depending on the targeted tissue. In addition, intraocular injections are currently the only way for proteins, such as monoclonal antibodies (mAbs) to be administered to the eye. Among intraocular injections, intravitreal and subretinal administrations are the most commonly performed and are usually either solutions or suspensions (50).

Intravitreal injections are currently the primary choice in targeting the posterior segment due to the direct injection into the vitreous. This administration route bypasses the anterior segment and blood ocular barriers, reaching the VH and insuring 100% vitreal bioavailability of the drug (7). Once the drug has dispersed in the vitreous, the administered drug can then distribute to the retina or to the anterior segment by entering the AH through diffusion or permeation through the BAB. Although intravitreal administration is highly effective, the procedure is invasive in comparison to topical administration. Furthermore, when treatment involves injecting macromolecules such as mAbs in the eye, repeated dose administration is needed. This is due to the relatively short half-life of macromolecules in the vitreous,

requiring frequent dosing to sustain effective drug levels. The repeated intraocular injections may lead to potential harm to eye structures and could result in conditions such as retinal detachment, cataracts, hyperaemia and endophthalmitis (14,57,58). Short-term complications include inflammation, intraocular infection, haemorrhage and vitreous incarceration (59).

Additionally, subretinal administration targets the retina, which is notoriously difficult to reach for systemically administered drugs due to the RPE, which is the outer layer of the BRB. Subretinal injection overcomes this and allows effective retinal drug delivery. Similar to intravitreal administration, subretinal injections are invasive and could lead to retinal detachment or damage (7,50). Furthermore, subretinal injections are commonly used for gene and cell therapies (56).

1.4.4 Periocular

Periocular administration routes commonly used in ophthalmology practice are subconjunctival and sub-Tenon injections (60). However, other periocular routes such as posterior juxtascleral, peribulbar, retrobulbar also exist, although they are less frequently performed (50) (see Fig. 1.3). These administration routes use the ocular anatomical structures to their advantage to deliver the drug effectively. For example, the subconjunctival space can expand and act as a type of depot for drugs, targeting either the anterior or posterior segment of the eye (61,62). Additionally, the sclera has a relatively large surface area (16.3 cm^2), which can be useful in drug delivery (63).

When drugs are delivered through subconjunctival injection, the drug is able to bypass the corneal and conjunctival barriers. This type of injection is done to bypass the conjunctival epithelial barrier, which is an important barrier for hydrophilic drugs (7). Once injected beneath the conjunctival membrane, the drug is able to permeate through the sclera, which exhibits greater permeability than the cornea, and reach the posterior segment (64). This administration route is seen as less invasive than intravitreal injections (14,65).

The sub-Tenon route involves injecting drugs into the space between Tenon's capsule and the sclera, either with a needle or preferably a blunt probe. Advantages of this administration route are the fewer complications and the lack of sharp needles during the procedure. This route is commonly applied when administering ocular anaesthesia (14,66,67).

1.5 CHALLENGES IN OCULAR DRUG DEVELOPMENT

As previously mentioned, the complex anatomy of the eye poses a significant challenge for ocular drug delivery. Moreover, the various ocular barriers and compartments complicate the quantification of drugs in a specific site of

action in the eye. Similarly, tracking the permeation of ocular drugs across different ocular compartments presents difficulties (68). Furthermore, difficulties in the sampling of ocular fluids also form an important obstacle in ocular PK research.

Animal models, as discussed above, provide a good insight into human ocular PK. However, these experiments incur costs and time. The ethical issues surrounding animal testing is also a factor that should be considered in ocular drug development.

Several administration routes are employed to bypass the ocular barriers, however, there are still several considerable drawbacks that are present. As previously mentioned, topical administration results in a low bioavailability, but is the most commonly used administration route. Intraocular injections are able to provide a higher and almost complete bioavailability but are invasive and not risk free. Periocular injections could provide alternatives, but some ocular drugs, for example proteins, can only be administered intraocularly (68).

Furthermore, sustaining a sufficiently high drug concentration within the eye poses a challenge. This is due to several physiological barriers that hinder the distribution within the eyes, such as the endothelial membrane between the corneal stroma and the AH. A possible solution to this might be controlled release formulations (68). However, testing and evaluating different dosage formulations, often with subtle differences, *in vivo* is not feasible in animal models.

1.6 WHAT IS PBPK?

Physiologically based pharmacokinetic modelling (PBPK) is a computational tool that attempts to describe drug flow throughout the body. It achieves this by integrating physicochemical and *in vitro* PK data into a mechanistic model, wherein various organs are represented as compartments (69,70). This enables the modelling of absorption, distribution, and clearance for a specific drug and organ and across the whole body. PBPK provides insight into the specific and relevant absorption, distribution, metabolism, and excretion (ADME) processes influencing *in vivo* PK, thereby aiding rational decision-making in drug development. PBPK models, which are constructed in such a way that the models reflect physiological processes *in vivo*, are able to predict overall PK behaviour, while also providing insight into the relevant PK processes (70). Essentially, PBPK serves as a surrogate for *in vivo* physiological processes.

PBPK models differ from classical PK models, such as compartmental and non-compartmental PK analysis. PBPK modelling enables the prediction of drug concentration-time profiles by integrating *in vitro* PK data, physicochemical drug characteristics and physiological parameters, before any animal or human studies have been done. In contrast,

classical PK modelling derives PK parameters based on observed *in vivo* data, often from human studies, without explicitly incorporating physiological processes. Therefore, the parameters present in PBPK model equations are directly associated with the physiological and biochemical processes in the body or organ (70).

Advantages in using PBPK models include easy inter-species scaling, use of a mechanistic and realistic modelling approach, and the flexibility of the models to incorporate several scenarios and even pathological conditions (71). By using PBPK models, the amount of preclinical animal studies requested by regulatory agencies can be significantly limited and potentially waived (68). Therefore, the cost and drug attrition rate in drug development can be significantly reduced (72). Furthermore, PBPK models can, together with other models, such as Quantitative Systems Pharmacology models, aid in early clinical stages with prospective dose selection (56).

1.6.1 GastroPlus®

GastroPlus® is a simulation software that employs mechanistic PBPK modelling to simulate how drugs are absorbed and predict their PK after various administration routes, including intravenous, gastrointestinal, ocular and pulmonary, in both human and animals. This software is commonly utilized in drug development and offers numerous applications. These include predicting first-in-human and animal doses, simulating drug-drug interactions, assessing food effect differences, and various other applications relevant to drug development (73).

The Ocular Compartmental Absorption & Transit (OCAT™) model is the ocular model within GastroPlus®. This ocular PBPK model allows insight in the disposition of drugs in the various, otherwise unreachable or hard to sample ocular tissues or compartments (74). The various ocular segments, tissues and barriers relevant to ocular drug delivery are integrated in this intricate model, reflecting the physiology of the eye. Additionally, this ocular model offers various dosage forms and administration routes, including topical, intravitreal, and subconjunctival options, as well as the potential for implants and controlled-release formulations. Furthermore, a full PBPK model can also be linked to the OCAT™ model, allowing the PK modelling of systemically administered drugs, whether oral or parenteral.

It should be noted that the OCAT™ model does not support the modelling of macromolecules such as proteins or peptides. Therefore, only the properties of small molecules can be used as input in this model.

By using this model, it is possible to study the absorption and PK of drug compounds within a virtual eye and make minor adjustments between simulations to gain a comprehensive understanding of the PK under different scenarios. This great advantage is otherwise unattainable when studying the PK in animal eyes, as it requires considerable time,

financial resources, and raises ethical concerns regarding animal welfare and protection laws. Additionally, the OCAT™ model has been employed in drug formulation to enhance the bioavailability of ocular dosage forms such as topical ointments to the extent possible (74).

1.7 IMPORTANCE OF RESEARCH

As the world population continues to age, the incidence of age-related ocular diseases is on the rise. There is a growing interest in ocular drug development, especially in protein drugs, due to the nature of the age-related illnesses. The urgency to develop ocular drugs swiftly and efficiently stems from the critical role vision plays in individuals' daily lives, as well as the imperative to enhance their overall quality of life.

To address this need, a profound comprehension of ocular PK is paramount in ocular drug development to understand the underlying mechanisms that hinder drug delivery to the eye. The use of PBPK modelling offers unique advantages to both accelerate and refine the process of ocular drug development.

2 OBJECTIVES

The aim of this thesis is to provide an in-depth overview of the PK of drugs in the eye through a literature study, explaining the PK processes in detail in both the anterior and posterior segment of the eye. As topical and intravitreal administrations are in practice the most used administration routes, the PK of these routes will be used as a guideline to give structure to the overview of anterior and posterior ocular PK, respectively. Relevant factors influencing the PK in the eye, such as physicochemical properties of drugs, and disease state will also be covered. This way, a comprehensive understanding of the key PK processes in the eye will be obtained.

As PBPK modelling is nowadays being implemented more frequently in drug development, the exploration of its application in ocular PK offers interesting insights. This exploration includes the opportunity to grasp the complex interplay of the physiological and drug-dependent factors that influence drug disposition in ocular tissues in a mechanistic manner. By experimenting with the OCAT™ model in GastroPlus®, a deeper understanding of how PBPK modelling functions in the context of ocular PK will be provided, while implementing the insights from the literature study. It is important to note that while PBPK modelling is a complex topic in itself, the objective of this thesis is not to perfect the modelling, but to use it as a tool to enhance the understanding of ocular PK and of the PBPK modelling itself.

There will be two PBPK modelling experiments conducted using the OCAT™ model. Firstly, PK modelling of intravitreal triamcinolone acetonide (TA) in rabbit will be conducted. After sufficient model optimization to the observed data from rabbit studies in the literature, the physiology of the rabbit model will then be translated to the human situation. The output of both models will then be compared to the observed values from literature in both species, which is an interesting way to get a grasp of the ability of the OCAT™ model to do interspecies extrapolation.

Secondly, existing, validated OCAT™ models for levofloxacin and gatifloxacin as topical solutions by Le Merdy et al. will be utilized (75). Although these models have been validated for topical solutions, it would be interesting to explore whether they can also predict the PK of the same drugs after oral administration.

3 METHODS

3.1 LITERATURE SEARCH AND SCREENING

The literature search was done by using the search term *("ocular pharmacokinetics")* in PubMed®. This search term was used on the 19th of February and yielded 309 results, covering a wide range of topics within the field. The records relevant to this thesis, which were for the most part comprehensive reviews and chapters of ocular pharmacology books, have been included in this literature search. Additionally, the snowballing method was used to identify further records relevant to this thesis. This was done by consulting the reference lists of the existing records.

Another literature search was conducted to collect as many human studies as possible with sufficient PK data. Human PK studies of ocular drugs have been identified from two databases, namely PubMed® and Web of Science™.

The search terms used have the following structure: *("Pharmacokinetics") AND ("eye") AND ("matrix concentration") AND ("concentration") AND ("human*") AND ("administration route").* The full search term can be found in the supplementary data. This search term was entered in both databases on the 4th of March 2024. Before screening, duplicates were removed using Rayyan (76,77). Further screening of the articles was done based on the title and abstract, together with the first set of exclusion criteria that are listed in the PRISMA diagram (Fig. 3.1), found in the supplementary.

The studies were then further screened based on topical or intravitreal administration. The amount of PK data was considered, for example, the presence of a graph with several data points was preferred in comparison to one table with a mean concentration at one timepoint. If data on the physiochemical properties of the compound were difficult to find, online or in the available literature, these studies were also excluded. The compatibility of the compound with the OCAT™ model was considered, as macromolecules are not supported as input. Further, fixed drug combinations and measurements in biological matrices that are not compatible with the OCAT™ model, such as tear fluid, have also been excluded from the selection. Lastly, the presence of a rabbit ocular PK study with the same ocular drug and administration route was the last exclusion criterion.

The search term *("triamcinolone acetonide") AND ("intravitreal") AND ("rabbit*") AND ("aqueous humor")* yielded 18 results. Among these results, the rabbit PK study of Arie et al. was chosen, due to the available PK data of the

compound in several ocular tissues and plasma (78). After screening, the study by Beer et al. observing AH concentrations of intravitreal triamcinolone in human was included for the first experiment (79).

For the second experiment, another set of exclusion criteria was used. As the model by Le Merdy et al. is validated for levofloxacin and gatifloxacin, studies that observed other drugs were excluded. If the administration route was a topical solution, this was also excluded, as the model is already validated for topical solutions. The inclusion of plasma values in the study was preferable. The selected human PK studies on oral levofloxacin and gatifloxacin were extracted, namely the study by Pea et al. and by Rajpal et al., respectively (80,81). These studies were chosen because they applied to the oral administration route and because of the analysed biological matrix (AH, VH and plasma).

3.2 PBPK MODELLING IN GASTROPLUS®

GastroPlus® (version 9.9 Simulation Plus Inc., Lancaster, CA, USA) was used for the simulation of all three compounds (TA, levofloxacin, and gatifloxacin). A total of four models will be discussed in the following section. PK data from the four studies were extracted using WebPlotDigitizer (82).

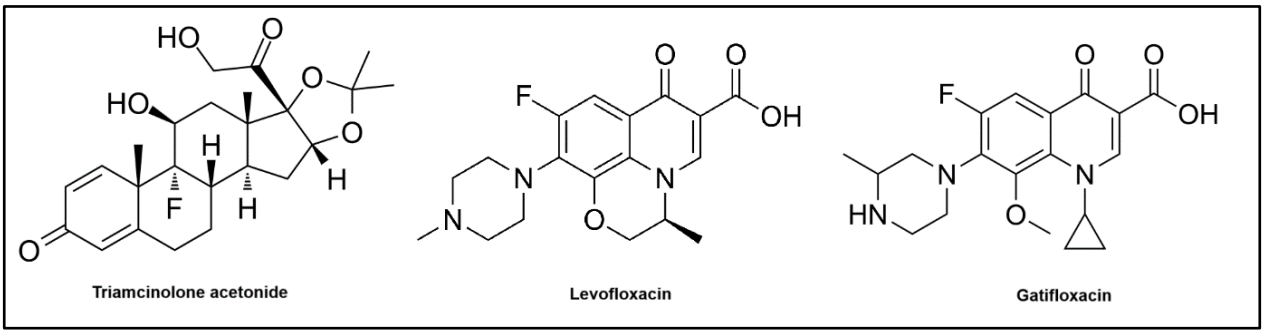

Figure 3.2: Chemical structures of TA, levofloxacin, and gatifloxacin. Adapted from References (83–85).

3.2.1 Triamcinolone acetonide (TA)

The AH, VH and plasma data points were collected and loaded into GastroPlus® version 9.9. Physicochemical properties of TA that were found in the available literature were adapted in the program, such as Log P and the solubility of the drug. PK parameters such as clearance (CL) and volume of distribution (V_d) were taken from the literature and implemented as a compartmental model. The dosage form was set to a vitreal implant instead of a vitreal suspension to better characterize the depot behaviour the suspension exhibits in the VH.

To model the slow, controlled release of the drug, a Weibull function was fitted. A Weibull function is a way to describe the release from a controlled release dosage form in an empirical way. The Weibull function was fitted based on the plasma concentration values from the rabbit study. The full Weibull function for this rabbit model can be found in the supplementary. Afterwards, initial simulations were performed with the default parameters of the OCAT™ model. After manual optimization of permeability parameters in the ocular model, the simulated curves were acceptably fit to the observed values. The model fit was achieved by incrementally increasing the permeability of the VH compartment in the ocular rabbit model until further increases no longer improved the alignment between the observed and predicted values.

This model was then duplicated, with the adjusted parameters saved. The physiology of the model was then changed from rabbit to human. The overall model was kept the same, except for the initial dose and dose volume, which was adjusted to match the human study by Beer et al. (79). Additionally, the Weibull function was fitted to the human observed values. Furthermore, a few parameters were adjusted, namely the solubility of the drug and the permeability in the VH compartment. Table 3 in the supplementary includes all the adjusted parameters in both rabbit and human model.

To determine and compare the fold difference between the predicted values and the observed values of the rabbit model, calculations have been made. These calculations include ratios of the TA levels in the different matrices, namely AH/VH, AH/plasma, and VH/plasma for both the predicted and observed TA values. These ratios were based on the last three data points, measured at 338 h, 669 h, and 1341 h. The datapoint in 2182 h was excluded, due to the lack of observed values in the other matrices at this time point. The predicted and observed ratios were then compared.

Additionally, to compare the rabbit and human model, ratios of the observed TA levels to predicted TA levels in AH were calculated for both models. Due to the lack of observed data in the VH and plasma for the human model, TA concentration ratios in AH were calculated. The human AH ratios were calculated using the measured data points at 243 h, 412 h, and 748 h from the human study. The rabbit AH ratios were calculated based on the observed time points from the rabbit study. These AH ratios were then compared to assess differences in scale of the observed to predicted ratios between the two species.

3.2.2 Oral levofloxacin and gatifloxacin

The topical ocular models by Le Merdy et al. were implemented in GastroPlus® (75). This was done by adapting the physiological and specific drug parameters of both models from both the article and the supplementary data. To confirm the accuracy of the implemented models, simulations were conducted using the data from the studies cited in the article for model validation. The outputs of the implemented models were then compared to those by Le Merdy et al. Once these outputs matched, the implemented models of levofloxacin and gatifloxacin were deemed ready for use. The dosage forms, initial doses and dose regimens were adapted to match the articles. Additionally, the PBPK physiology of both models was adjusted to match the patients from the studies, specifically, the mean age, body weight, and renal clearance.

4 RESULTS

4.1 OCULAR PK

The PK-processes of ophthalmic drugs in the eye will be discussed thoroughly in the following sections. There is a distinction made between the anterior and posterior segment in the eye, due to the significant difference in PK in both segments.

4.1.1 PK of the anterior segment (topical administration)

When focusing on treating conditions in the anterior eye segment, the main methods of administering drugs include topical application, subconjunctival and intracameral injections (17). However, it is worth noting that topical application remains the most commonly used method, as mentioned earlier. The PK of topically administered drugs will be further discussed in this section since it is the most common administration route for ocular drugs. Topically administered drugs target anterior ocular tissues such as the cornea and its different layers, conjunctiva, sclera, and the ICB (7).

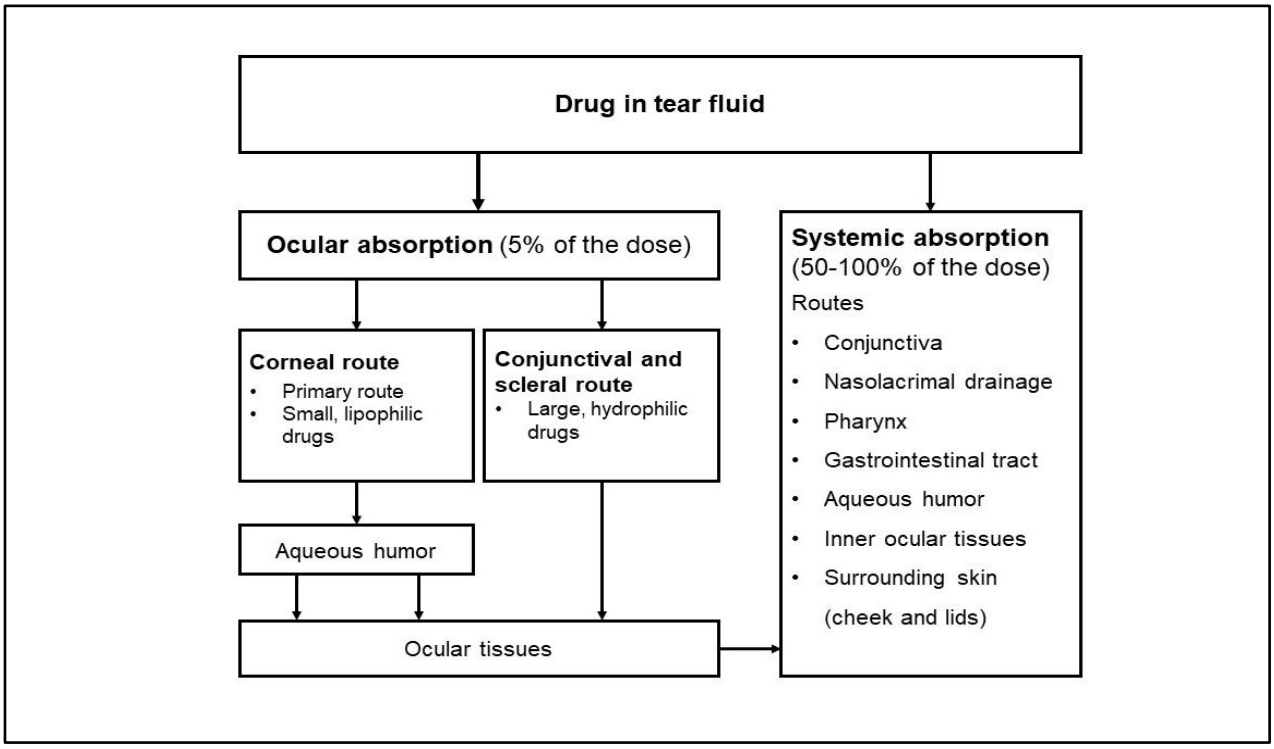

Figure 4.1: Schematic representation of absorption routes of topically administered drugs. Adapted from Reference (49).

ABSORPTION

The most crucial factor influencing the PK in the anterior segment of the eye is absorption. Once an ophthalmic drug is administered topically, it mixes with the tear fluid present on the surface of the eye. From there, the drug can be subjected to either ocular absorption or systemic absorption. Topically administered drugs are known to have a low bioavailability, as only 5% of the drug dose is able to absorb into the ocular tissues. This low bioavailability is due to several factors: precorneal surface loss, corneal and non-corneal barriers, and certain drug properties (17,49).

Precorneal surface loss is the loss of drug from the surface of the eye before actual absorption of the drug in the cornea. Factors such as tear turnover, blinking, nasolacrimal drainage, and clearance via systemic circulation after nasolacrimal drainage, contribute to precorneal surface loss. In humans, the tear turnover rate is 0.5-2.2 µL/min, which is relatively fast when considering the normal tear volume (7-9 µL). Additionally, patients tend to blink after administration as a reflex that could then lead to the drug to flow out of the eye and into the surrounding skin (usually the cheek and lids). The conjunctival sac is a small pocket where the inner and outer segments of the eyelids meet, in other words, the space between the eyelid and the eye. The conjunctival sac and tear film can hold about 30 µL of fluid without overflowing. When administering eye drops for example, the volume of the precorneal tear film increases abruptly after instillation, leading to tear volumes of 40 µL to 70 µL depending on the drug formulation. This leads to reflex blinking and rapid drainage of the tear fluid into the nasolacrimal duct, where the majority of administered drug is either cleared systemically or lost by flowing into the stomach (i.e. nasolacrimal drainage) (14,17). After nasolacrimal drainage, systemic absorption of the drug via the gastrointestinal tract is possible.

Additionally, the drug can also be systemically absorbed to a lesser extent through the mucosal walls of the pharynx (49). Drugs can be absorbed systemically through the conjunctiva as well. However, this route is deemed nonproductive, indicating that this route causes significant drug loss out of the eye through systemic absorption. This is due to the conjunctival blood capillaries and lymphatics present in the conjunctiva (see Fig. 4.2). The drug, once absorbed through these vessels, can more extensively enter the systemic circulation, resulting in reduced ocular bioavailability (7,14).

Then, the remaining drug (which is about 5% of the original dose at this point) can be absorbed in the eye through different ocular absorption routes, either corneal or non-corneal (see Fig. 4.1). The primary absorption route for topical ophthalmic drugs is penetration through the cornea. Drugs that are small and lipophilic benefit the most from this

absorption route. The cornea is composed of several layers, however, the three relevant layers for drug absorption are the corneal epithelium, stroma and endothelium. Together, these layers of the cornea form a significant barrier to drug absorption.

As previously mentioned, the corneal epithelium consists of several cell layers. These comprise of a basal layer composed of columnar cells, followed by two to three layers of wing cells, and lastly, one or two outermost layers of squamous superficial cells. As previously mentioned, the squamous cells have tight junctions, that restrict the absorption of hydrophilic drugs, favouring the transcellular permeation of lipophilic compounds. The basal and wing cells consist of wider, intercellular spaces (17). Drugs are able to permeate through the corneal epithelium using two pathways, namely the transcellular pathway and the paracellular pathway, both based on passive diffusion. The former is optimally used by lipophilic drugs. These drugs have a higher affinity for the lipophilic corneal epithelium, resulting in retention of the drug. This can then be released gradually from the epithelial cells to the stroma (14). The latter pathway is preferred by hydrophilic compounds with a small molecular weight (<350 Da), passing through the wide intercellular spaces of the basal and wing cells (16,17,86). The stroma is a thick, fibrous, and hydrophilic tissue, allowing hydrophilic compounds with a small molecular weight to permeate through with minimal resistance. The corneal endothelium is a more leaky, partially hydrophilic barrier, which can be attributed to the large, intercellular junctions present between the cells. This leads to the limited penetration of lipophilic compounds and compounds with a higher molecular weight. Hence, it can be deduced that for highly hydrophilic drugs, the corneal epithelium serves as the rate-limiting barrier, while highly lipophilic drugs face rate-limiting partitioning from the corneal epithelium to the hydrophilic stroma (86). Additionally, the cornea forms an impermeable barrier for macromolecules, such as protein drugs and other compounds with a high molecular weight (17).

Key drug properties affecting corneal absorption are lipophilicity, molecular size, aqueous solubility, and ionization. A study on the corneal permeability of several different steroids and β-blockers was conducted and the optimal Log P range was around 2-3 (18). An exploratory analysis by Prausnitz and Noonan compiled the apparent corneal permeability (P_{app}) of almost 150 compounds from 40 different studies, primarily small molecules (87). This analysis compiled the permeability of drugs through the full cornea, including the corneal epithelium, stroma, and endothelium, as well as the permeability in individual corneal layers separately. However, due to the largely limited availability of literature, data on corneal epithelium alone were absent in the analysis. Additionally, the scleral and

conjunctival permeability of several compounds were included. This literature analysis provides an extensive database of permeability data from both *in vivo* and *in vitro* studies conducted on several ocular tissues across different species. The study concludes that corneal permeability is dependent on the partition coefficient (Log D at experimental pH) and molecular size (87). Although this study examined only a limited amount of macromolecules, it can be concluded that macromolecules are not able to cross the cornea, due to the fact that generally, compounds with a radius larger than 10 Å cannot permeate through the cornea at a measurable rate (88).

Another result of this study is that polar surface area (PSA), representing the polar portion of the molecule and correlating with aqueous solubility, also influences corneal permeability. Higher PSA values indicate greater hydrophilicity, resulting in lower corneal permeability. Stromal permeability, where passive diffusion plays a major role, was found to be negatively correlated with molecular weight and radius in the analysis (87). The cut off point for the diffusion of molecules through the stroma is 500 kDa (89). Although it is a hydrophilic tissue, there was no correlation found between stromal permeability and lipophilicity (Log P or Log D) based on the limited number of molecules analysed. However, the authors found that the stroma alone exhibits relatively high permeability compared to the full cornea. Based on this observation, the authors concluded that the stroma primarily forms a barrier for highly lipophilic compounds, which can readily cross the corneal epithelium and endothelium, thus making it one of the rate-limiting layers. Overall, the hydrophilic nature and diffusion-based transport mechanism of the stroma are what determines permeability through this layer (17,87).

The permeability of the corneal endothelium was shown to have a large dependence on lipophilicity and molecular radius. Like the cornea, the permeability of the corneal endothelium increases with a moderate increase in lipophilicity. However, it should be noted that data on highly lipophilic compounds were lacking when analysing the permeability of this barrier, therefore further investigation on the barrier properties was not possible. Furthermore, due to the large, intercellular junctions present in the corneal endothelium, a strong inversely proportional correlation with molecular radius was observed with the included compounds, which was expected. Based on this study, it is difficult to compare the permeability of this corneal layer to that of the epithelium, as permeability data of the corneal epithelium alone are very scarce (87).

Typically, unionized drugs exhibit greater permeability compared to ionized drug (90). Ionizable drugs are able to permeate through the corneal epithelium depending on their degree of ionisation and ionic charge (90). The corneal epithelium consists of ionizable sites that can be protonated, with an isoelectric point of 3.2 (91). As a result, positively charged compounds penetrate the corneal epithelium more readily compared to negatively charged ions. At physiological pH (approximately 7.40), the corneal epithelium is negatively charged, allowing selective passage of positively charged molecules, while repelling negative ions, via ion exchange (91). Conversely, the corneal epithelium exhibits a positive charge at pH levels below 3.2. Administering an ocular drug product with a pH level lower than the isoelectric point (which is significantly lower than physiological pH) can cause irritation due to increased acidity. Such formulations are not used in clinical settings, as topical formulations are strictly prepared to match physiological pH. Therefore, in clinical practice , the corneal epithelium limits the permeation of negatively charged molecules (86).

Overall, considering the gathered data from this analysis, the corneal epithelium predominantly acts as the primary barrier for molecule transport across the cornea. Drugs that can easily permeate the corneal epithelium are small molecules with optimal lipophilicity, while the corneal stroma may present a hindrance to larger molecules (17,87).

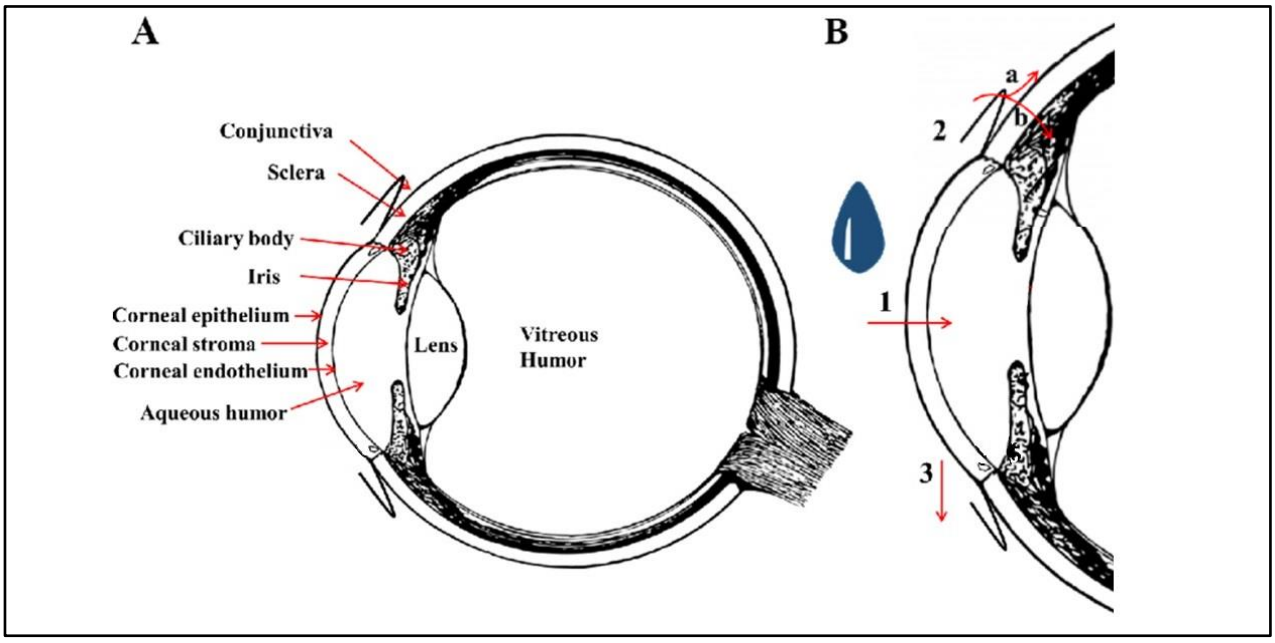

Figure 4.2: Schematic representation of the anatomy of the eye (A) and corneal and non-corneal absorption routes (B). 1: corneal absorption; 2a: conjunctival absorption into conjunctival blood vessels and lymphatics (systemic absorption); 2b: conjunctival and scleral absorption; 3: precorneal surface loss. Adapted from Reference (92).

Non-corneal routes of absorption consist of the permeation of topically instilled drugs through the conjunctiva and scleral layers (see Fig. 4.2). These routes are utilized when drugs have suboptimal properties, such as poor corneal permeability due to high hydrophilicity or when they are too large in radius or molecular weight (17,93). Therefore, the contribution of non-corneal absorption routes is dependent on the drug properties of the topical drug. The scleral permeability is strongly dependent on molecular radius, similar to the stroma alone, and no apparent correlation with partition coefficient was observed (87). Compared to the full cornea, the sclera has a relatively high permeability, motivating researchers to explore transscleral drug delivery for topical drugs that need to reach the back of the eye, such as the retina (63,94). The conjunctiva, the membrane that covers the anterior sclera and the posterior part of the eyelids, is structurally composed of a stratified columnar epithelium and lamina propria (12,17). Similar to the corneal epithelium, the conjunctival epithelium consists of tight junctions but has wider intercellular spaces in comparison. The permeability of hydrophilic drugs is therefore greater in the conjunctiva. Based on the limited studies available on conjunctival permeability, it appears that lipophilicity of a drug does not significantly influence conjunctival permeability, whereas increasing molecular weight tends to have a negative effect. While macromolecules appear to exhibit limited permeability through the conjunctiva, additional studies are necessary to determine the exact impact of macromolecular size on conjunctival permeability (17,87).

It can therefore be concluded that in most cases, depending on the drug and the absorption pathway, the permeability of the drug through the corneal epithelium is what defines absorption of topically administered ophthalmic drugs.

DISTRIBUTION

After absorption through the cornea, the topically administered drug readily enters the AH. Subsequently, it can then distribute to the neighbouring ocular tissues, including those that are targets of the drug, including the ICB, lens, choroid-retina, and vitreous (16,17). Drugs that make use of the non-corneal absorption route will reach the uveal tract, which is the middle layer of the wall of the eye and is made up of the choroid and ICB, and the vitreous, bypassing the AH (17,95).

There are several factors that influence the distribution rate and extent of an ocular drug in the AH. These include diffusion in the matrix, protein binding and the surrounding ocular tissue. Physicochemical properties of drugs are able to influence most of these factors, these properties include molecular weight, solubility, and lipophilicity (17).

The volume of distribution (V_d) of an ocular drug in the AH is measurable when administered intracamerally. There is however a lack of data on the PK of intracamerally administered drugs. The V_d of specific ocular drugs following intracameral injection in rabbits was compiled and analysed by Durairaj et al. (17). This analysis included estimated PK parameters and relevant physicochemical properties of the drugs. Overall, the V_d of the different drugs was found to be between 2-10 times larger than the AH volume in rabbits, which is approximately 0.3 mL. The analysis revealed a correlation between plasma protein binding and V_d, indicating that drugs known for high protein binding in plasma tended to exhibit low V_d in the AH. Highly plasma protein-bound drugs typically have prolonged plasma half-lives ($t_{1/2}$), and this tendency extends to their $t_{1/2}$ estimated in the AH. Correlations between V_d and molecular weight or Log P were not observed in this analysis (17). In plasma, high molecular weight drugs tend to have a lower V_d, due to their inability to permeate through tissues (96). In contrast, lipophilic drugs tend to have a higher V_d in plasma, as they are able to diffuse easily through lipid bilayers, allowing them to leave the systemic circulation and distribute to adipose tissues (97). Although these trends are well known in plasma, additional studies are necessary to clarify the correlations between molecular weight, lipophilicity and V_d in the AH.

Topically administered drugs are first subjected to protein binding when mixed with the tear fluid, which as mentioned above, has a rapid turnover rate. Due to the protein binding, only free drug in the tear fluid is available to permeate through the cornea. Subsequently, the drug undergoes more binding in both the cornea and the AH. The most important drug-binding protein is melanin, which is present in high levels in the ICB. Important to note is that the protein concentration in the AH is vastly different compared to plasma. Approximately, the protein levels present in the AH are 200 times lower than in plasma. Protein levels in the AH may however increase due to certain disease states, which will be discussed in a later chapter (17).

By measuring the concentration of a topically administered drug in the AH, the V_d can be estimated (17). However, to investigate if the drug effectively reaches the target tissue, the surrounding ocular tissue concentrations must be measured. As previously mentioned, this is not commonly done due to its highly invasive nature and the significant number of animals required for such studies. Scarcity in such data is frequent, however, there are a few PK studies that report the drug levels in both the AH and relevant ocular tissues in rabbits. The area under the curve (AUC) ratios of drugs in ocular tissues and the AH from these studies have been compiled by Durairaj, along with relevant drug properties (17). Overall, the relative drug levels in the cornea were higher than the AH and in other tissues, like the ICB, which is expected since the cornea is an important barrier for drugs. Higher molecular weight compounds

exhibited significantly higher corneal drug levels, as the diffusion through the cornea is difficult for such compounds. Additionally, the relative drug level is higher in the ICB than in the AH. There are several explanations to this phenomenon, namely the high porosity, vascularity, and surface area of the rabbit iris, allowing the drug to thoroughly penetrate into the ICB (24).

Furthermore, drugs with high affinity to melanin can distribute more extensively to the ICB, as it consists of a high amount of melanin pigment. Generally, it can be concluded that all basic and lipophilic compounds are able to bind to melanin (98). Due to their affinity to melanin, drugs such as brimonidine and levobetaxolol, known to bind to melanin, exhibit significantly higher relative drug exposure in the ICB compared to the cornea and AH (17). Thus, melanin binding may alter the amount of drug that reaches its target tissue, depending on their affinity to melanin. Melanin binding does this by preventing the whole drug dose from reaching its target site and binding to receptors in ocular tissues, therefore leading to a reduced fraction of the drug dose at the site of action. Consequently, this leads to the bound drug being released at a very slow rate, similar to a drug depot (7,64,99). Due to the lowered drug dose at the target site, larger doses might be needed to achieve a therapeutic response (100). While a link has been made between drug accumulation due to melanin binding and potential ocular toxicity, it is important to note that melanin binding alone does not necessarily indicate ocular toxicity (98).

Non-corneal absorption routes could also contribute to the higher drug levels in the ICB. Some drugs, based on their physicochemical properties, may prefer the non-corneal absorption route via the conjunctival or scleral pathways. This leads to drugs reaching the ICB tissues, avoiding the AH (17). Further, experiments were conducted using beta-blockers with different lipophilicities, along with sucrose and inulin measured in various ocular tissues. These experiments concluded that the outer layer of the sclera allows easier penetration of hydrophilic compounds compared to the cornea (93). Additionally, the conjunctival and scleral permeability is estimated to be 15-25 times higher than that of the cornea, and is not influenced by molecular size (101).

METABOLISM

Drug metabolizing enzymes in the eye, like those found elsewhere in the body, have the capability to convert drugs into their metabolites, which can result in a decrease in ocular drug bioavailability (14). However, this is not the case for prodrugs, which become active following metabolism. The various ocular drug metabolizing enzymes and

transporters are well-described in the available literature. These enzymes primarily belong to families such as oxidoreductases (including cytochrome P450, aldehyde oxidase, ketone reductase), hydrolases (such as aminopeptidases and esterases), and conjugating enzymes such as transferases. For more information on the specific enzymes present in the ocular tissues and their role in ocular drug delivery, a thorough summary by Attar et al. is available (102). Only a set of clinically significant enzymes and transporters with *in vivo* evidence will be discussed (17).

Table 4.1: List of clinically significant enzymes. List adapted from Reference (17).

Enzyme	Enzyme class	Location in eye	Substrate	Source
Aldehyde oxidase	Oxidoreductase	Cornea, conjunctiva, ICB	Brimonidine	(103)
NADPH-dependent ketone reductase	Oxidoreductase	Corneal epithelium, conjunctiva, ICB, lens	Levobunolol	(104)
Arylesterase	Esterase (hydrolase)	Cornea, ICB	Dipivefrin	(105)
Aminopeptidase	Hydrolase	Corneal epithelium, ICB, conjuctiva, and AH	Bimatoprost	(106,107)

Aldehyde oxidase is an enzyme expressed in the cornea, conjunctiva and ICB (102,108). Brimonidine is a drug metabolized by this enzyme and its metabolites were measured in the cornea, conjunctiva and ICB of the rabbit after topical administration (103). This is due to the expression of aldehyde oxidase in these tissues. Another oxidoreductase is NADPH-dependent ketone reductase. This enzyme is present in ocular tissues such as the corneal epithelium, conjunctiva, ICB and the lens. Topically instilled levobunolol comprises a ketone functional group and is subjected to metabolism by NADPH-dependent ketone reductase, resulting in its metabolite, dihydrolevobunolol. This metabolite has the same activity as the parent compound, but with a much longer $t_{1/2}$ and therefore higher exposure in the ocular tissues (104).

Esterases are a subgroup of the hydrolases and are present in various ocular tissues, with characterized differences in expression in different ocular tissues (17). Dipivefrin is an anti-glaucoma prodrug and is known to be primarily metabolized in the rabbit cornea. This conclusion has been challenged, due to the higher metabolism rate in the rabbit

ICB. Additionally, when dipivefrin was co-administered with an esterase inhibitor, there was no observed decrease in therapeutic activity. It was hypothesized that arylesterase could play a role in the metabolism of dipivefrin, which consists of a phenol ester, and this could explain why the used esterase inhibitor (which was a cholinesterase inhibitor), did not affect the therapeutic activity of the compound. Moreover, other esterases, such as acetyl-, butyryl-, and carboxylesterases, were found to be present in the rabbit eye (17,105)

Another identified enzyme group are the aminopeptidases, which are able to cleave peptides and proteins (109). Their activity was characterized in several albino rabbit ocular tissues and fluids, namely the corneal epithelium, ICB, conjunctiva, and AH. Bimatoprost is a prostamide analogue and after topical administration, its metabolite bimatoprost acid, was detected in the AH and cornea (106). This observation shows the influence of aminopeptidase in the metabolism of bimatoprost (107).

The presence of drug transporters has been identified in both the anterior and the posterior segment of the eye. These transporters could be either influx or efflux transporters, the former acting as a drug carrier and facilitating absorption and distribution, and the latter acting as a barrier for drug permeation. Although clinical relevance of ocular drug transporters is not yet confirmed in the available literature, it is expected that ocular transporters have an important role in determining ocular drug levels (14). For example, influx transporters have been used as a tool to increase the bioavailability of prodrugs via specific transporter-targeted modifications (7). Additionally, various ophthalmic drugs have been observed to interact with ocular drug transporters, examples of such drugs include anti-glaucoma drugs, antibiotics, anti-inflammatory drugs, H1-receptorantagonists, etc. (110).

In the anterior segment, efflux transporters are present in both the cornea and conjunctival tissues, where they play a protective role by expelling xenobiotics that have diffused into ocular cells. One well-described efflux transporter in ophthalmic literature is P-gp. A study using erythromycin observed a significant increase in corneal drug levels of erythromycin when testosterone, a P-gp inhibitor, was present. This indicates the influence of P-gp on the drug's corneal exposure (17,111).

ELIMINATION

Most of the topical drug dose is lost due to precorneal surface loss, as mentioned earlier. The drug dose will then absorb systemically after draining through the nasolacrimal duct. After systemic absorption, the majority of the drug metabolizes and gets eliminated through the known systemic pathways (liver metabolism, renal excretion, etc.) (17).

Additionally, the metabolic breakdown of ocular drugs in the anterior ocular tissues contributes to drug elimination. The presence of metabolic enzymes in the these tissues has been well-documented in the available literature, along with clinical evidence of several enzymes affecting drug levels in the ocular tissues, as mentioned in the preceding section.

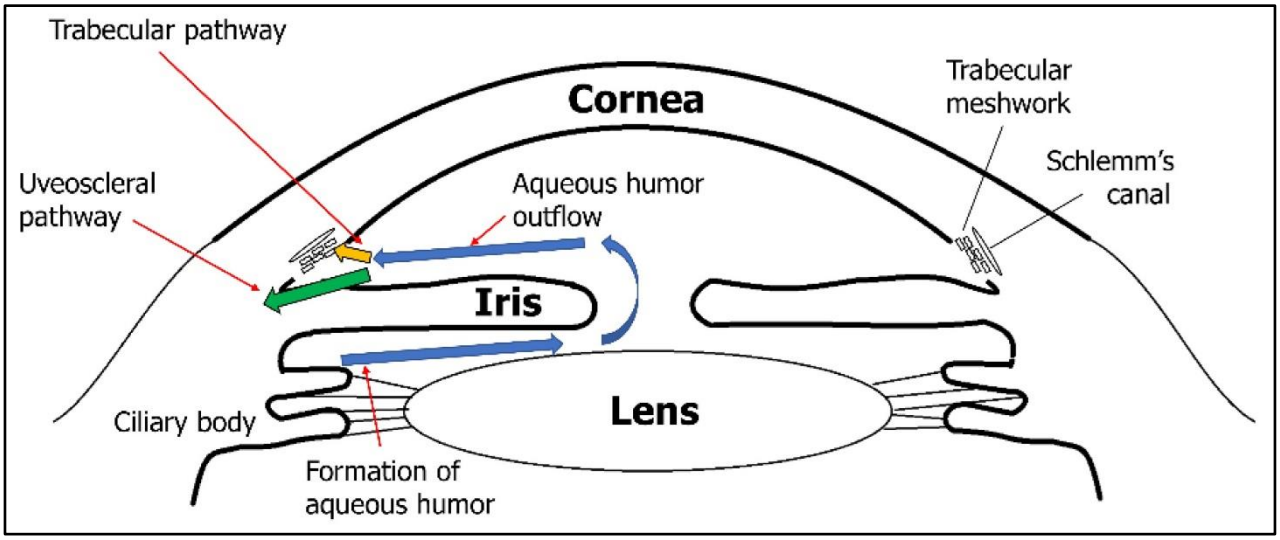

Figure 4.3: Schematic illustration of the uveoscleral pathway (green arrow) and trabecular pathway (yellow arrow). Adapted from Reference (112).

The small fraction of drug that is able to absorb through the cornea and into the AH is also subject to elimination. This elimination from the AH can occur via two pathways, namely through the turnover of the AH through the anterior chamber angle and Schlemm's canal (trabecular pathway), and through systemic absorption via the venous capillaries in the anterior uvea, which consists of the ICB and choroid (uveoscleral pathway) (see Fig. 4.3) (24). The anterior chamber angle is a structure between the cornea and the iris, consisting of the trabecular meshwork. The main function of the trabecular meshwork is to drain AH from the anterior chamber (9). This is deemed the conventional path for AH drainage, as it is responsible for drainage of the majority of the AH volume (113). Through the trabecular meshwork, the AH can flow into the Schlemm's canal, which eventually reaches conjunctival and episcleral veins present on the scleral surface (114). The second pathway is deemed unconventional, as the small, remaining fraction of AH is drained via this route and involves passive flow of fluid along a pressure gradient (115).

The AH turnover rate in rabbit is equal to 1.5% of the volume of the anterior chamber per minute, resulting in a $t_{1/2}$ of 46 minutes. This rapid turnover rate leads to the faster clearance of hydrophilic drugs in comparison to lipophilic drugs. This claim can be further supported by observing the elimination $t_{1/2}$ of both intracamerally and topically administered drugs. Some of the intracamerally administered drugs, compiled by Durairaj earlier, exhibited a prolonged $t_{1/2}$ in the AH due to their high protein binding properties. However, these same drugs are also lipophilic in nature. The compiled hydrophilic drugs had a $t_{1/2}$ in the range of 0.4 and 0.69 h, while the most lipophilic drug (Log P 4.11), which was at the same time the most protein-bound drug, had a $t_{1/2}$ of 1.55 h in the AH (17).

In the case of topically administered drugs, Durairaj found, after compiling studies and data, that highly lipophilic drugs (Log P > 2) exhibit a longer elimination $t_{1/2}$ in the cornea compared to other tissues. This phenomenon can be explained by the fact that the corneal stroma is hydrophilic and acts as a barrier for highly lipophilic drugs. Consequently, the clearance of these drugs from the cornea is slowed down due to their slow partitioning through the stroma (17).

4.1.2 PK of the posterior segment (intravitreal administration)

The posterior segment of the eye consists of various structures, such as the sclera, choroid, RPE, retina, optic nerve, and VH. It is known that administering drugs to the posterior segment is much more challenging than the anterior segment, due to the enforced ocular structures (sclera, RPE, and blood capillary endothelial cell walls) and the blood and lymph circulation (1). Among various administration routes, intravitreal administration persists as the pragmatic option for delivering drugs to the posterior segment, despite its invasive nature and potential complications, as mentioned earlier (2). Intravitreal injection is often the sole route for delivering several specific drug classes to the posterior segment, including antibiotics, vascular endothelial growth factor (VEGF)-inhibitors, and steroids (3).

The PK of intravitreally administered drugs is less explored and relatively poorly understood in comparison to topical PK (33). However, following sections of this thesis will provide insight into the observed PK processes after intravitreal administration, as documented in the available literature. Overall, there are two primary factors that influence the PK of intravitreal drugs: the distribution in the VH and the clearance of these drugs (3).

ABSORPTION

As mentioned earlier, the PK of intravitreally administered drugs is not heavily reliant on absorption, unlike topical drugs, due to the direct injection of the drug into the VH. Therefore, it can be concluded that nearly all of the drug dose reaches the VH and there is no significant absorption phase (17).

DISTRIBUTION

The distribution of drugs through the VH is a crucial PK process, as drugs must reach the targeted ocular tissue in the posterior segment. Factors that influence distribution in the VH consist of the drug's diffusion through the vitreous, convective flow, and possible interactions between drugs and elements of the VH. Furthermore, depending on the target site, posterior ocular barriers can be a hindrance to drugs (116). Additionally, the injection itself affects the distribution by creating a small channel through the VH upon injection. Upon needle removal, it leaves behind a pathway with low resistance for drug passage, as it alters the integrity of the VH (117).

The drug will, after injection into the vitreous chamber, diffuse through the VH until the target tissue is reached. VH drug diffusion happens due to the change in concentration gradient at the site of injection, providing a driving force for the drug molecules to diffuse throughout the VH until an equilibrium is reached. The rate at which this happens is reliant on the physicochemical properties of the drug and the vitreous retention effect. The latter refers to the tendency of drugs to be retained within the VH due to the structural properties of the VH itself. The former refers to drug physicochemical properties that influence the vitreous retention effect and in turn the diffusion through the VH, namely molecular weight and the net charge of the drug (33).

Due to the presence of collagen fibres and glycosaminoglycans, the VH forms a mesh network that can act as a barrier where drugs need to diffuse through (33,34). Regarding molecular weight, it can be concluded that compounds with a low molecular weight have high diffusion coefficients, due to the easy diffusion through the mesh network. This was observed with compounds such as fluorescein, glycerol, and mannitol, with a molecular weight ranging from 92-332 Da (118,119). Conversely, the diffusion of compounds with a high molecular weight, such as fluorescein isothiocyanate-labelled dextrans (4-80 kDa), through the VH can be limited (120). Additionally, the net charge of the intravitreal drug can influence its diffusion through the VH, since the VH itself is a polymeric network with a negative charge. This factor is more limiting for the diffusion of cationic drugs, due to the potential electrostatic interaction with the negatively charged components of the VH, such as hyaluronic acid (121,122). This leads to VH retention, thereby decreasing the VH diffusion of the drug. However, the overall effect on the PK of cationic drugs is not clarified (33).

Convection or convective flow in the eye involves the movement of a fraction of volume from the AH through the VH towards the retina (37,123–125). This phenomenon occurs as a result of pressure and temperature differences between

the anterior chamber and the retinal surface (33,37). The influence of this process on the drug diffusion through the VH is largely dependent on the diffusion coefficient in the VH (124). Drugs that exhibit high VH diffusion, such as low molecular weight drugs, are not influenced by convection due to the substantial movement through the VH. The influence of convection on drugs with a low diffusion coefficient is not currently concluded, however, the consensus generally holds that convective flow exerts a significant impact when drug diffusion is low and the flow is increased (33). Using computational models, Park et al. supported the claim that drugs with high VH diffusion (1×10^{-5} cm^2/s) are not affected by convection. However, this process becomes relevant for drugs with low VH diffusion (1×10^{-7} cm^2/s), especially at increased vitreous outflow rate, which can occur in in pathophysiologic conditions such as glaucoma (123,124). Additionally, Del Amo et al. recently investigated the influence of convection on drug diffusion in the VH through a literature review. The authors concluded that there is no evidence to date proving that convection plays a major role in drug distribution in the VH (35).

Similar to the AH and plasma, the VH consists of proteins. Proteomic analysis of human VH samples found protein concentrations of 4.7 ± 1.2 µg/mL, with albumin being the most abundant protein (60-70% of total VH protein) (126,127). Despite the relatively low protein concentration, drugs are able to interact with these vitreous proteins, leading to a decrease in free drug after binding. This could potentially affect the pharmacological effect. Additionally, drug diffusion through the VH could be limited, increasing the drug residence time in the VH (33). Another relevant protein is melanin, which is not only present on the ICB, but is also distributed on the choroid and RPE (17). Additionally, studies have shown that there are regional differences in melanin amount in both human and in several animal species (128,129). Regardless of their administration route, ophthalmic drugs will unavoidably encounter melanin-pigmented tissues, either during their distribution or elimination in the eye. Melanin binding is becoming increasingly interesting for research due to its influence on ocular drug disposition (17). A handful of studies have observed intravitreal PK of selected drugs in both pigmented and non-pigmented animals. An overview of these studies is provided by Durairaj et al. (130). Based on the data collected, it appears that the vitreal $t_{1/2}$ of drugs administered to pigmented rabbits is significantly longer than that in albino rabbits. Due to the limited data, a correlation with specific physicochemical properties could not be established (17).

Liquefaction is a process that influences both VH drug diffusion and convection. This process is the degeneration process of the VH, which is linked to aging. VH is present in both liquid and gel phases, however, as individuals age,

the liquid phase of the VH increases while the gel phase decreases due to the disruption of the mesh network (37,131). Liquefaction could lead to higher drug diffusion through the VH, especially to the drugs with limited VH diffusion, as the disrupted mesh provides more freedom of movement for the drugs to diffuse through the VH. Although liquefaction does not directly influence drug elimination, the increased diffusion caused by liquefaction could in turn increase drug elimination in the posterior segment. The higher the liquefaction of the VH, the more the diffusion of drugs resembles that of in water (35). Conversely, an increase in convective flow is also linked to liquefaction and the loss of vitreous homogeneity due to aging (89). Based on the given data, the same dosing scheme is possibly not applicable for patients of all age groups, as it could lead to either overdose of insufficient pharmacological effect. However, the influence of liquefaction on intravitreal PK has not yet been extensively documented in the literature (47,58).

The target site of ocular drugs in the posterior compartment depends on the specific disease, as the drug target could be located in the VH itself, or be other tissues such as the retina or the choroid (33). Understanding the distribution of drugs from the VH to the surrounding tissues is crucial for effective treatment. Del Amo et al. have compiled all available studies with small molecule intravitreal PK data in rabbit and determined the intravitreal V_d of each compound (132). The authors found that the V_d was overall relatively constant, as 80% of the Vd calculated ranged from 1.2-2.2 mL in rabbit. Additionally, the authors concluded that the V_d was not dependent on the drug's molecular weight and lipophilicity as the compounds analysed had different structures and therefore different drug properties. Because the calculated V_d of the drugs was similar to the anatomical volume of the rabbit VH, it was suggested that the surrounding tissues in the posterior segment have an insignificant influence on the V_d. Two factors explain this observation: firstly, the anatomical size of the posterior segment tissues is minor compared to that of the VH volume. Secondly, most of the surrounding tissues (excluding the lens) also take part in the elimination of drugs into the systemic circulation. The back diffusion from these tissues to the VH is therefore minimal and does not contribute significantly to the V_d (35).

The BAB and posterior BRB are important barriers surrounding the vitreous. The inner BRB enables the diffusion of small molecules with a size less than 2 nm. Similarly, the RPE, which is part of the outer BRB, is a compact layer of cells located between the photoreceptors and choroid. Molecular size and lipophilicity are factors that influence the

permeability of drugs through the RPE. Upon reaching the choroid, drug diffusion occurs rapidly due to the increased permeability of the choroid, facilitating fast removal of the drug into the blood circulation (35).

In the available literature, it has been demonstrated that the retina is consistently provided with nutrients by the influx-and-efflux carriers present on the BRB (37,38,110). Main carrier families detected in the BRB include P-gp, BCRP, some MRP, and organic anion transporting polypeptides (OATPs) (133). Generally, drugs can act as substrates for active transporters, this is also applicable to those in the BRB. Although the functionality of a few transporters and their substrates have been studied, unravelling the role of these transporters to the PK of intravitreal drugs is far from complete (35).

Initially, the high concentration of intravitreal drugs in the VH can saturate transporters present on the BRB, which in turn reduces their impact on drug distribution (133). As drug concentrations decrease over time, transporter saturation decreases as well, indicating that the impact of transporters could theoretically increase over time. However, due to the limited *in vivo* studies and the overall low expression levels of these transporters in the BRB, their overall impact on drug distribution remains unclear (35). Recent reviews on transporter expression in the BRB have been published (134,135), however, it should be noted that the expression of some transporters in specific ocular tissues was hypothesized without confirmation from *in vivo* studies (35). Because of this, possible differences in transporter expression between species also remains unclear (35).

The presence of these transporters can be both advantageous and disadvantageous for drug distribution. If the target site of the drug is the choroid, these active transporters can facilitate the drug's access to the choroid, even those drugs that typically cannot permeate the BRB. Conversely, if the target site is located anterior to the retina or is the retina itself, the active transporters will contribute to the drug's elimination (33).

Regarding drug diffusion in the VH, it is suggested that active transport has minimal influence on drug distribution, as suggested with the help of quantitative structure-property relationship (QSPR) modelling and quantitative proteomics studies (130,132,136). On the other hand, PK model simulations by Vellonen et al. have shown that RPE efflux proteins are able to limit drug access to the RPE or increase its elimination in specific situations (133). These include low drug concentration in the VH, high transporter affinity, and high transporter expression. This way, active transporters may significantly affect drug distribution and in turn drug bioavailability (35,133).

METABOLISM

So far, the metabolism of drugs in the vitreous has not been extensively documented (33). There are studies available on intravitreal metabolism, but these are primarily focused on the identification of drug metabolizing enzymes in the vitreous, not on their influence on intravitreal PK (33,137). Similar to the anterior segment, esterases and peptidases have been characterized in rabbit VH (138). Additionally, these enzymes have been utilized to develop prodrugs. An example of this is ganciclovir esters, that are metabolized by esterases into ganciclovir after intravitreal injection (138). Furthermore, the presence of drug-metabolizing enzymes has been characterized in the ocular tissues succeeding drug elimination from the VH, such as the retina and ICB (139).

ELIMINATION

Two primary pathways for drug elimination from the vitreous exist: anterior and posterior clearance (33). The primary choice in either elimination pathway is heavily dependent on the drug's molecular and physicochemical properties (17).

The anterior clearance route consists of the clearance of intravitreally administered drugs towards the anterior segment of the eye. This happens by diffusion from the VH through the lens and ICB, entering the anterior chamber. Afterwards, the drugs can then be removed together with the AH through the trabecular and uveoscleral pathways (38). The main driving force of anterior clearance of intravitreal drugs is the rapid turnover of the AH into the anterior chamber (37). The range of drugs that can be eliminated via this clearance pathway is broad, as they can easily diffuse through the hyaloid membrane, which consists of a layer of collagen that separates the VH from the rest of the eye (33). Drugs that are large and hydrophilic are typically eliminated through this route, as the retina forms an impermeable barrier for these drugs (37). Additionally, the anterior clearance of drugs with a large molecular size has been well-documented (58,140,141). From these studies, it can be concluded that molecular size is inversely correlated with the vitreous elimination rate, leading to a longer vitreous $t_{1/2}$ of intravitreal drugs (33). Data from experimental studies suggest that drugs eliminated through the anterior pathway generally have longer elimination $t_{1/2}$ than those eliminated through the posterior route. Durairaj compiled the AUC of selected drugs in the VH, AH, and retina or choroid (17). An exponential correlation between the vitreal $t_{1/2}$ and the AUC ratio of the AH and VH was observed, namely that the drugs that had a low AH/VH ratio had a shorter vitreal $t_{1/2}$. This means that drugs that are mainly eliminated through the anterior pathway exhibit a longer vitreal $t_{1/2}$. A clear correlation between lipophilicity and vitreal $t_{1/2}$ could not be made due to the limited data. However, there was an overall trend that indicates that an increase in lipophilicity

leads to a decrease in vitreal $t_{1/2}$. This is expected, as adequately lipophilic compounds are able to penetrate the tight junctions present on the RPE (17). In conclusion, hydrophilic drugs with a large molecular size are mainly eliminated through the anterior route, with AH outflow being the main elimination mechanism (17,33,130).

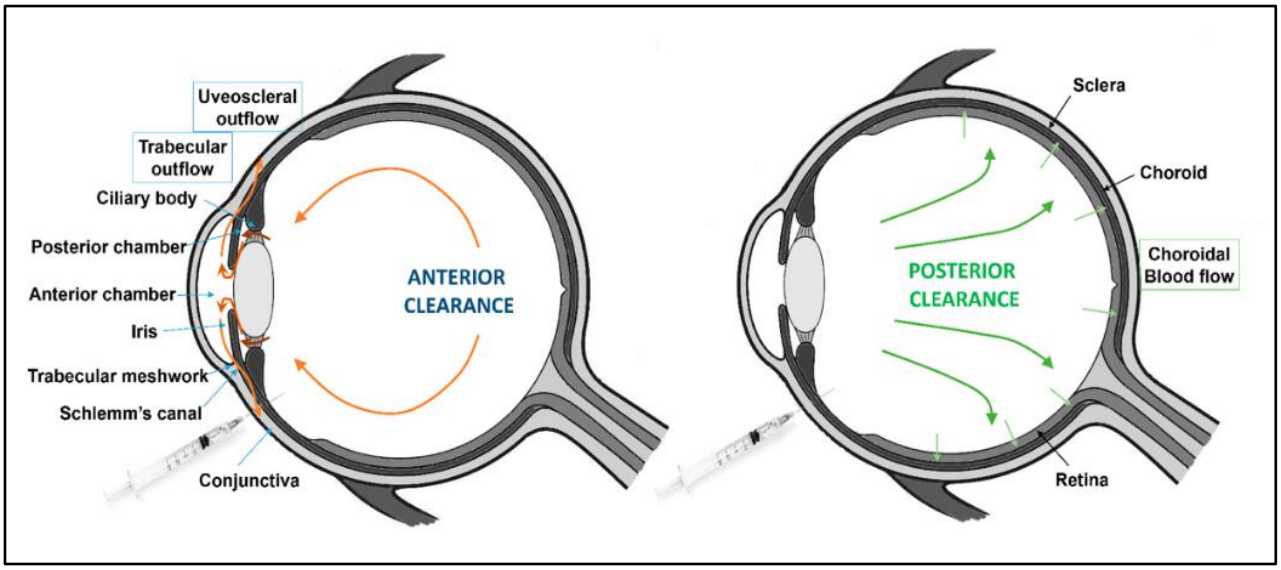

Figure 4.4: Schematic representation of the vitreal clearance pathways. Adapted from Reference (33).

The posterior clearance route concerns the permeation of intravitreal drugs through the retina. The drugs are then cleared via choroidal blood flow. Contrary to the anterior route, the drugs eliminated by the posterior route have a shorter $t_{1/2}$. This is attributed to the large surface area, tissue partitioning, and the active transport mechanisms (17). Durairaj et al. observed a correlation between vitreal $t_{1/2}$ of drugs and specific physicochemical properties by developing correlation models. The major parameters that influence vitreal $t_{1/2}$ include molecular size, lipophilicity, and the dose/solubility ratio at pH 7.4, coined as dose number (130). Once a drug dose surpasses its vitreous solubility, it leads to the formation of a depot or suspension, leading to a gradual release of drug over time. As expected, when the dose number was greater than 1, the apparent elimination $t_{1/2}$ was greatly increased. Additionally, submodels were also developed for several subsets depending on dosage form, animal model, ionization state, and molecule size. It was found that for macromolecules, the vitreal $t_{1/2}$ is primarily positively influenced by molecular weight. For ionizable compounds, the major property affecting vitreal $t_{1/2}$ is lipophilicity (17,130). As a result, it can be concluded that the posterior pathway is mainly utilized by small, lipophilic molecules, as they can readily cross the retina, either through

paracellular and/or transcellular diffusion. Additionally, choroidal blood flow is what drives this elimination pathway (17,33,130).

4.1.3 Influence of disease state on ocular PK

Although various disease states are known to alter the PK of ocular drugs, its influence is not widely explored in the ocular drug delivery field (17). These ocular diseases may cause physiological conditions that in turn may affect ocular bioavailability of these drugs (14).

Since the anterior surface of the eye is in direct contact with the environment, both the anterior and posterior segments of the eye are susceptible to infections caused by bacteria, viruses, and fungi (142). Bacterial endophthalmitis is a common inflammatory disease that, even with proper therapeutic measures, often leads to irreversible vision impairment as it inflames tissues in the posterior segment of the eye, often damaging the retina (143). A study by Barza et al. observed the PK of several cephalosporins in normal and infected eyes of rabbits after intravitreal and subconjunctival injections (144). It was observed that after repeated subconjunctival administration in infected eyes, the drug exposure in the vitreous was 2-9 times greater than non-infected eyes. This observation, however, is most likely due to the repeated dosing, rather than inflammation. After single intravitreal injection of ceftizoxime and ceftriaxone in infected eyes, the $t_{1/2}$ of these drugs was longer in comparison to normal eyes. This results from the inflammation of the ocular tissues, which damages transport pumps and results in extended $t_{1/2}$ of drugs that are mainly cleared through the posterior clearance route. This conclusion is further supported by two studies that observed the drug exposure of intravitreal ketorolac in normal and inflamed rabbit eyes respectively (145,146). On the other hand, Coco et al. observed that the vitreal $t_{1/2}$ of vancomycin after intravitreal injection in inflamed rabbit eyes (13.6 h) was decreased in comparison to normal eyes (62.3 h) (147). The observed heightened clearance was linked to the increased permeability resulting from the disruption of the BRB (17).

Breakdown or loss of integrity of the BRB is one of the prevalent complications of diabetic retinopathy and age-related macular degeneration (148,149). Cheruvu et al. conducted an animal study to examine the impact of diabetes on the PK of periocular celecoxib. For this study, diabetes-induced rats were examined after dose administration. The results of this study showed that in the diabetic rats, a 2.4-3.5-fold higher BRB leakage was observed in comparison to the control rats. Due to the BRB disruption, celecoxib drug levels were 1.5-2 times higher in the VH and retina (150). Additionally, another study by Shen et al. examined the ocular PK of intravitreal brimonidine and dexamethasone in rabbits and monkeys with BRB breakdown (149). However, in this study, the drug levels are lower in the observed

ocular tissues of the rabbits with BRB breakdown than those of the normal rabbits. The monkeys with BRB breakdown exhibit another result, namely that the only tissue that had lower drug exposure was the central retina/choroid region, where laser lesions were applied to induce choroidal neovascularization (CNV), which leads to BRB breakdown. Brimonidine and dexamethasone had significantly higher ocular tissue drug exposure in normal monkeys when compared to the diseased monkeys. In conclusion, the differences between the studies of Cheruvu and Shen can be attributed to several factors, namely the variations in species, induction methods of BRB breakdown, and the specific compound properties. These results do however support the increased clearance in animals due to BRB breakdown, leading to lower exposure in ocular tissues. Moreover, the study of Shen et al. highlighted the importance of compound- and disease-specific differences in ocular PK when extrapolating findings to other species (17).

Glaucoma is one of the most common ocular disorders globally that can cause vision impairment (1). One of the established risk factors for this ocular disease is an increased intraocular pressure, which causes impairment of the optic nerve, leading to atrophy of this nerve along with defects in field of vision (151,152). Since the vitreal convective flow depends on the pressure in the vitreous chamber, logically thinking, the increased intraocular pressure may possibly alter the flow rate (33). Computational models of the rabbit eye have been developed, with consideration of the possible influences of convection in both normal and pathological conditions (123,125). It has been observed that in pathologies that exhibit increased intraocular pressure, like glaucoma or retinal detachment, that convection increases (33). However, the influence of this increased convection on the PK of ocular drugs has not been clarified in the available literature.

Diabetic retinopathy has been shown to exhibit a small increase in protein concentration in the VH (127). Although this theoretically might lead to higher fractions of drug bound to protein, there are currently no available studies that cover the impact of this increase on the PK of drugs in the VH (153).

Del Amo et al. have, however, conducted a literature analysis to explore the influence of disease state on the PK in the posterior segment (35). The authors did this by collecting data from studies that compared posterior segment PK in diseased and healthy animals and then calculating and compiling relevant PK parameters. Results of this analysis found that there is no convincing evidence that BRB breakdown or damage would significantly alter the PK in the posterior segment. The changes of the PK parameters (CL, $t_{1/2,}$ AUC, and concentrations in several matrices) induced by

the posterior segment diseases showed less than 1.5-fold difference between healthy and diseased animals. Because no major changes were found, the authors concluded that PK studies conducted in healthy animals should be deemed valid, as the changes in PK would not cause significant changes in terms of dosing (35).

4.2 PBPK MODELLING

4.2.1 Triamcinolone acetonide (TA)

The final output of the rabbit model is shown in Fig. 4.5. The squares in the graph represent the observed TA levels from the study of Arie et al. (78). The solid lines depict the predictions of TA levels calculated by the rabbit model. The green, red, and blue elements indicate the observed and predicted TA levels in the VH, AH, and plasma, respectively. The TA concentrations are in ng/mL.

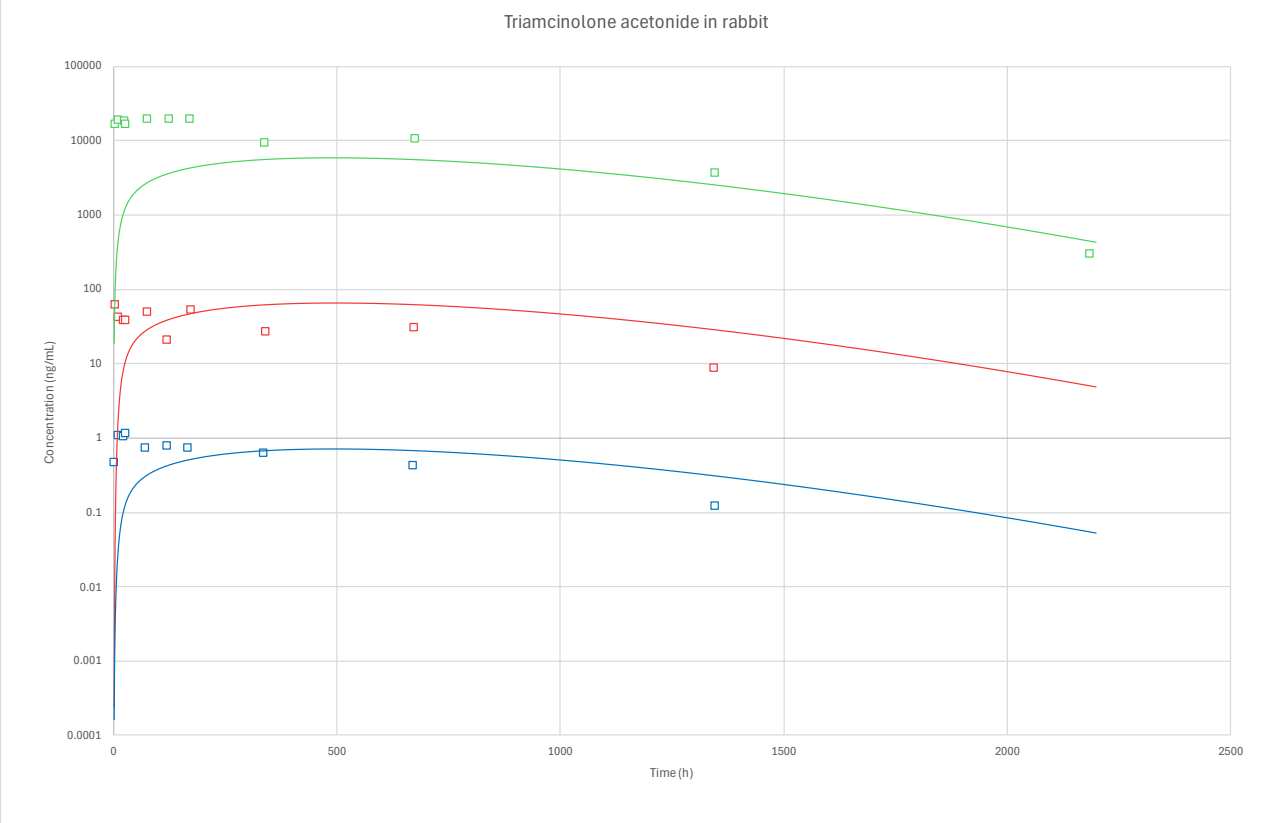

Figure 4.5: Simulation output of TA ocular rabbit model. Observed (squares) and predicted (solid line) values in VH (green), AH (red), and plasma (blue). Concentrations in ng/mL.

Subsequently, the physiology of the model was changed from rabbit to human, along with the solubility and VH permeability of TA. Fig. 4.6 shows the final simulation of the human model. The squares represent the observed TA levels in AH, obtained from the study by Beer et al. (79). The study did not include TA concentrations in VH and plasma. The green, red, and blue solid lines represent the predicted TA levels in the VH, AH, and plasma, respectively. The TA levels are in µg/mL.

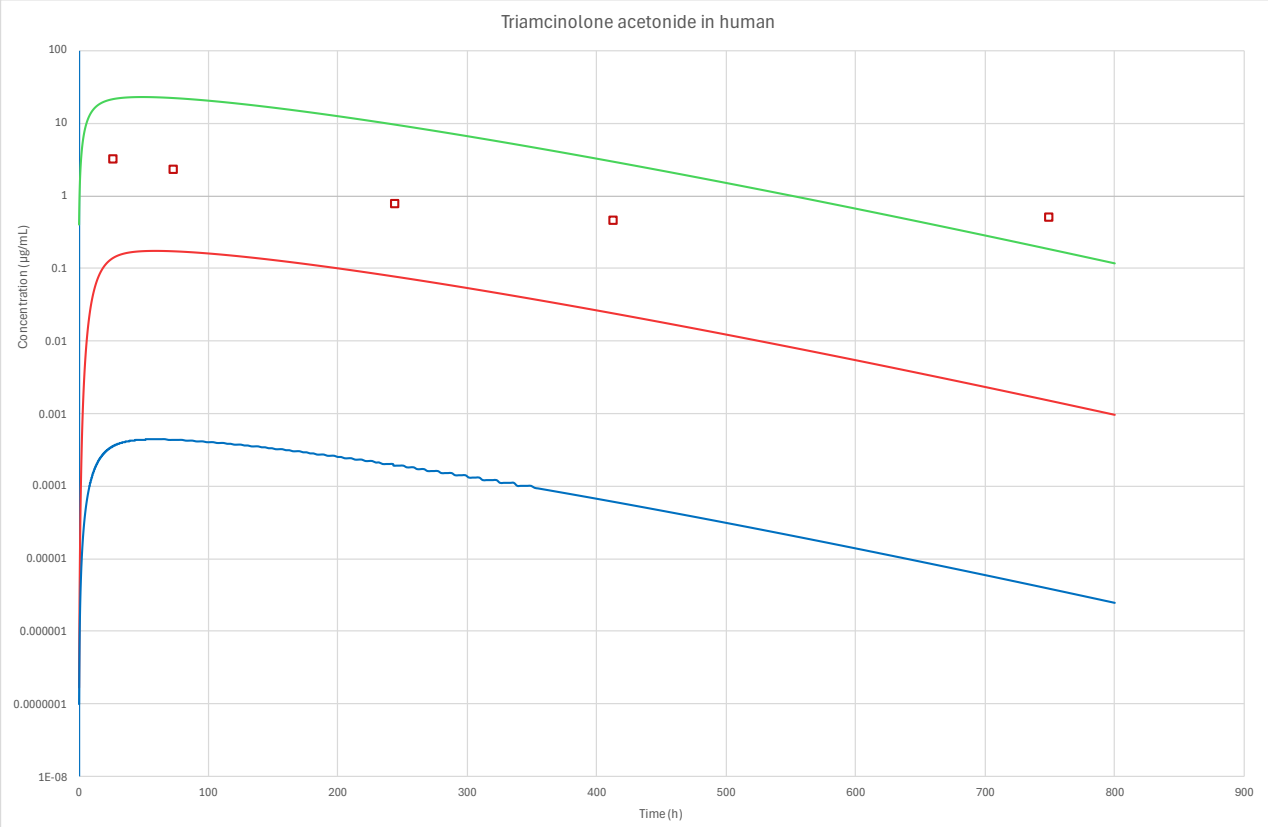

Figure 4.6: Simulation output of TA ocular human model. Observations (squares) and predictions (solid line) in the AH. Predictions in VH (green) and plasma (blue). Concentrations in µg/mL.

Table 4.2 shows the calculated ratios of the predicted and observed TA levels in different matrices in rabbit. These ratios include AH/VH, AH/plasma, and VH/plasma, and are based on the three last observed time points. This was done to compare the difference in scale between the predicted and observed ratios in rabbit in the different matrices.

Table 4.2: Calculated observed and predicted ratios in AH, VH, and plasma in rabbit.

	Time point	t = 338 h	t = 669 h	t = 1341 h
Observed	AH/VH	0.0028	0.0029	0.0024
	AH/plasma	43.2178	72.7358	71.2364
	VH/plasma	15273.7927	25292.1333	30255.3986
Predicted	AH/VH	0.0111	0.0112	0.0113
	AH/plasma	91.8394	92.0809	92.2809
	VH/plasma	8286.7794	8240.9117	8202.58275

Table 4.3 presents the calculations of the predicted to observed AH ratios in both rabbit and human. The ratios were calculated by taking the observed TA level at the last three time points and dividing them by the predicted TA level at these time points. This way, predictive ability of the rabbit and human model can be compared.

Table 4.3: Calculated AH ratios between observed and predicted values in AH in both rabbit and human.

	Timepoint	t = 338 h	t = 669 h	t = 1341 h
Rabbit	Observed	27.6316	31.6228	8.8965
	Predicted	61.9576	62.336	28.7981
	Ratio	0.446	0.5073	0.3089
Human	Timepoint	t = 243 h	t = 412 h	t = 748 h
	Observed	790.0000	470.0000	512.0000
	Predicted	76.6023	23.9007	1.5112
	Ratio	10.313	19.6647	338.801

4.2.2 Oral levofloxacin and gatifloxacin

The topical levofloxacin and gatifloxacin models by Le Merdy et al. were implemented in GastroPlus®. Subsequently, the drug dose; dose scheme, and physiology were adapted to match those of the studies by Pea et al. and Rajpal et al., respectively (80,81).

Fig. 4.7 shows the final output of the oral levofloxacin model. The squares represent the observed levofloxacin levels, while the solid lines present the predicted levels. The blue and red elements represent the predicted and observed levels in plasma and AH, respectively. Similarly, Fig. 4.8 presents the final output of the oral gatifloxacin model. The blue and green elements indicate the observed and predicted levels in plasma and VH, respectively.

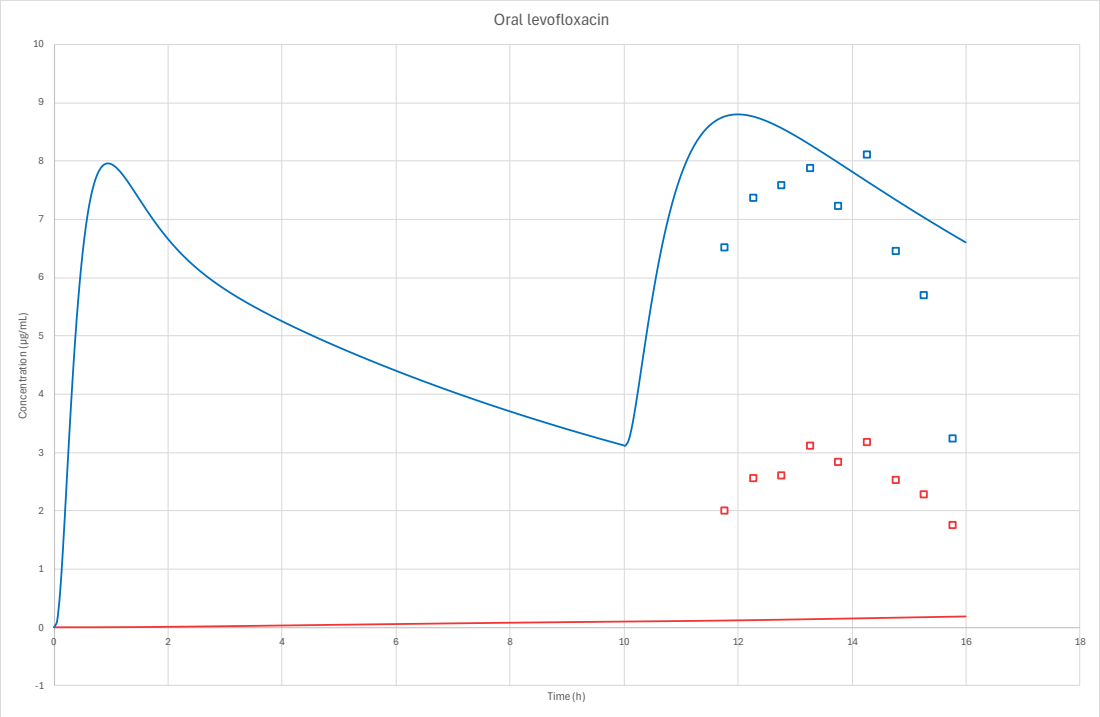

Figure 4.7: Simulation output of oral levofloxacin model. Observations (squares) and predictions (solid line) in the AH (red) and plasma (blue).

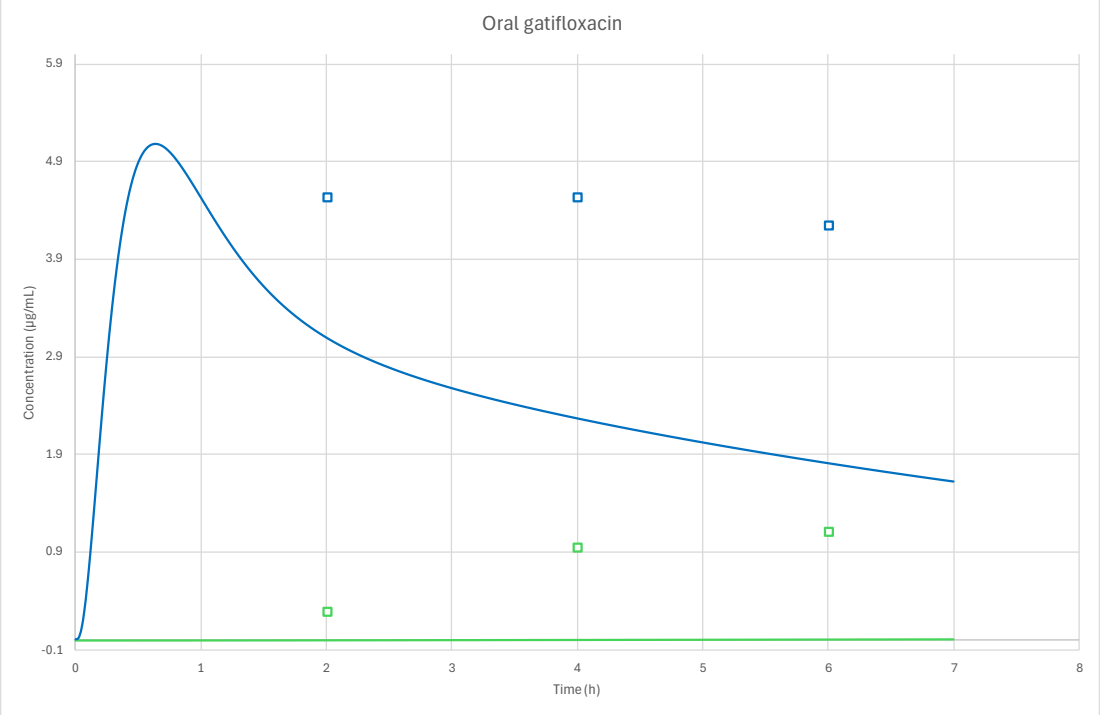

Figure 4.8: Simulation output of oral gatifloxacin model. Observations (squares) and predictions (solid line) in the VH (green) and plasma (blue).

5 DISCUSSION

5.1 LITERATURE SEARCH

5.1.1 General

To provide insight into the current understanding of how ocular PK works, a comprehensive overview of ocular PK through a literature search was performed. The PK in the anterior and posterior segment were explained in detail, following topical and intravitreal administration, respectively.

Topical ocular PK is heavily dependent on the absorption of the drug through the eye. These drugs can penetrate in the eye through the corneal or non-corneal absorption route. Corneal absorption is the main route, where the corneal epithelium is the main rate-limiting barrier for these drugs. Drugs that have optimal drug properties, namely sufficiently lipophilic small molecules, are able to penetrate through the corneal epithelium (and therefore the cornea as a whole). It can be concluded that macromolecules such as peptides and mAbs are not able to penetrate through the cornea after topical administration (14,17). Although anterior segment PK is relatively well described, the clinical relevance of ocular drug transporters has not yet been confirmed. These transporters, both influx and efflux, however, are deemed to have an important role in ocular PK (14).

In contrast, intravitreal ocular PK is mostly influenced by the distribution and clearance of the drug in the posterior segment of the eye. These drugs (both small molecules and macromolecules) are subjected to several physiological processes related to the drug diffusion in the VH, such as the VH mesh network and convection. Additionally, the physicochemical properties of a drug, such as molecular size, net charge, and lipophilicity, impact the diffusion and clearance processes. However, it is apparent that, in comparison to topical ocular PK, intravitreal PK is less explored, resulting in less clarity regarding the influence of certain physicochemical properties and physiological processes (33). These include cationic drugs, transporters, metabolism, and liquefaction, as mentioned earlier.

5.1.2 Similarities

Although PK of the anterior and posterior segment are significantly different, melanin binding is a topic that shows relevance in terms of drug exposure in the ocular tissues. Because of the large presence of this melanin in the ICB, RPE, choroid, and other ocular tissues, its influence is relevant in both segments (17,33). This physiological process of binding to melanin is an interesting topic for researchers, as it can alter the bioavailability of ocular drugs and prolong the duration of drug effect (7,64,99). Depending on the target site and the drug's affinity for melanin, melanin can

either increase (in melanin-rich tissue) or decrease the amount of drug dose that reaches the target tissue (17). Due to the latter effect, melanin binding has been associated with drug accumulation and ocular toxicity (mostly retinal toxicity), as higher doses might be needed. However, this is incorrect, as LeBlanc et al. concluded that the ocular toxicity previously attributed to melanin binding was actually due to the inherent toxicity of the studied drug. Therefore, the authors concluded that the affinity of a drug for melanin is not predictive of ocular (retinal) toxicity (98).

5.1.3 Differences and underexplored topics

Transporters present in ocular tissues seems to be a divided topic in the available literature, particularly between the two ocular segments. In the anterior segment, both influx and efflux transporters are well-identified and their expressions are thoroughly described in the literature (14,17). Several effects, from increasing bioavailability to reducing toxic exposure, have been well documented for various compounds. It should be noted that the clinical relevance of these transporters have not yet been fully clarified; however, researchers deem these transporters to play an important role in anterior segment PK (14). In contrast, transporters in the posterior segment have been mainly identified but not as thoroughly documented as those in the anterior segment (17,33). The role of these transporters on ocular PK is still underexplored, as a result of the limited number of *in vivo* studies done on this topic. Compared to anterior segment transporters, the role of posterior segment transporters appears to be minimal so far (35). Computational models and proteomics studies have shown that active transporters have minimal impact on drug distribution in the VH (130,132,136). However, simulations from another PK model show that RPE efflux proteins are able to limit drug access to this tissue only in specific situations (133). Overall, many aspects concerning posterior segment transporters still need to be clarified and validated *in vivo*.

Similarly to intravitreal PK, the influence of ocular diseases on ocular PK remains underexplored (17). Overall, studies on ocular inflammation in the anterior tissues seem to have the most promising data (144,145,147). Further, it seems that the number of studies on the impact of other anterior segment diseases on the PK is scarce. In the available literature, the focus seems to be more on posterior segment diseases. Studies that research the PK of specific drugs in normal and diseased rabbits and computational models provide insights in certain aspects of intravitreal PK, however, it is apparent that there are still several topics that need further investigation and clarification (33). As of the moment, it appears that the effect of disease state on intravitreal PK is relatively small, as suggested by Del Amo et al. (35).

5.1.4 Missing topics

The literature search provided a variety of topics in this field; however, one topic that was difficult to find was the translational aspect of ocular PK from rabbit to human. As rabbits and humans have slightly different ocular structures, it would have been interesting to see if anatomical and physiological differences between the two species could impact the PK of ocular drugs. Although the rabbit model is the most frequently used animal in ocular studies, its suitability as an ocular model for translation to the human eye has been questioned in the context of intravitreal drugs (47). Del Amo et al. conducted a systematic review of all studies on the PK of intravitreal drugs in rabbit and human, which led to following conclusion: the important PK parameters relevant for preclinical and clinical research (such as CL, Vd, and $t_{1/2}$) have a strong correlation, with similar absolute values (47). This leads the authors to conclude that the rabbit is a decent ocular model and translation to human should be reasonable (47). For anterior segment PK, however, the influence of these interspecies differences remains unclear. Worakul et al. reviewed several anatomical and physiological differences between rabbit and human in the anterior segment of the eye, carefully concluding that human clinical trials are necessary after collecting PK data in rabbits (49).

Furthermore, a general strategy for extrapolating ocular drug data from rabbits to humans could not be found in the literature. This would have been interesting to discuss in terms of dosing of ocular drugs from rabbits to human for example. Due to the lack of literature found on this matter, we are left to hypothesize that the extrapolation is done directly from rabbits to humans. The results of the systematic review by Del Amo et al. support this hypothesis, as the important PK parameters between rabbit and human have similar absolute values in the context of posterior segment PK (47). However, we make this claim cautiously, as relevant articles may have been overlooked during the literature search. Le Merdy et al. have developed an extrapolation strategy based on PBPK modelling using the OCAT™ model, where preclinical data in rabbits were used to validate a rabbit model. This model was then extrapolated to humans and successfully validated using human clinical data (75).

5.2 PBPK MODELLING

5.2.1 Triamcinolone acetonide (TA)

The PBPK modelling of TA in rabbit and human was conducted in GastroPlus®. Since no PBPK models of TA in the eye were available at the time of writing this thesis, this was an exploratory approach to understand ocular PK mechanistically, rather than to predict observed values accurately. It should be noted that the first part of the

predicted curves, which is the dissolution of TA in the VH, was not well captured in GastroPlus® (Fig 4.5). This is mostly due to the physicochemical properties of TA itself, which is highly lipophilic and is practically insoluble in water (saturated solubility 20 µg/mL) (78). TA acts as a depot inside the vitreous, while it is in reality an intravitreal suspension (154). The combination of being an intravitreal suspension while acting as a depot is difficult to model in GastroPlus®. When the model was first set to an intravitreal suspension dosage form, the dissolution part of the curve was relatively well-captured, however, it was unable to describe the slow release of the drug in the eye. Therefore, we have chosen for a controlled release intravitreal implant as dosage form, as the prolonged, gradual release of TA is an important aspect of its PK.

In the rabbit ocular model, the overall PK of TA in the three matrices exhibits a proportional behaviour. The highest drug levels in both observed concentrations and predictions were in the VH, which was expected as the drug dose was injected in this compartment. Additionally, it has been shown that the turnover rate of VH is slower compared to that in the AH, namely at most half of the rate in AH (155). The drug was, after dissolving in the VH, able to transfer from the VH to the AH by diffusion through the lens and ICB. Systemic levels of TA can be observed as the drug is subjected to both the anterior and posterior clearance pathways, as explained earlier in the literature search. Since TA is a small, lipophilic drug, it should be able to permeate through the retina relatively well after dissolution in the vitreous, with a relatively fast clearance into the systemic circulation (156–159). The rabbit ocular model was thus able to predict the observed concentrations relatively well, besides the first dissolution phase. The calculated ratios in Table 4.2 show that the predicted and observed values differ by no more than 5-fold.

Afterwards, the physiology was changed from rabbit to human, and the dose was adapted to that used in the human study (79). After the first simulation of the human model, it was apparent that the model significantly underpredicted the AH data points. Subsequently, the solubility of TA in the model was increased by a factor of 10. This increase in solubility can be attributed to the liquefaction of the VH in the patient population. Beer et al. stated that the patients in the study were elderly, leading us to believe that the liquefaction process was occurring in these patients, as it is an age-related process (33,79). Due to the decreased viscosity of the VH, we hypothesized that TA would have a higher solubility in this population. Additionally, the permeability of the VH was increased by a factor of 10^4. This parameter was altered because the patients in this study had macular oedema, which results from BRB breakdown (160). As previously mentioned, BRB breakdown can cause increased permeability in the posterior segment, supporting our

reasoning for changing this parameter. After increasing the solubility and VH permeability, the predicted AH levels came closer to the observed values, as shown in Fig. 4.6. Despite altering these parameters, the human model could not capture the observed values as well as the rabbit model. Table 4.3 shows the ratios of observed to predicted AH values of both models, further highlighting the difference in scale between the predictive ability.

Plasma and VH observations in the human study by Beer et al. are not available. However, the human model does predict the levels in plasma and VH in a similar fashion to that of rabbits. The absence of data in the other matrices limits our capability to validate this model. Additionally, the overall pattern of the human AH observations differs from the predictions, namely, the overall slope of the observations deviates. This could be due to the intra-subject variability in the patient population. Possible reasons for the intrasubject variability include differences in apparent V_d and or technical issues in delivering the exact dose of TA (4 mg) (79). Additionally, one of the 5 patients in the study had a prior vitrectomy, a procedure wherein the VH is (partially) removed (33,79). This led to this patient having a shorter $t_{1/2}$ than the other patients. The reasons for this shorter $t_{1/2}$ are unclear; however, they may include changes in the anterior or posterior clearance pathways due to surgical intervention, or differences in TA dissolution due to the altered movement in the vitreous after surgery (79).

This experiment demonstrates the translational aspect of PBPK modelling. The current human model could not successfully predict the human AH observations after adjusting the physiology. However, based on the predictions in rabbit, the predictions in human would exhibit a similar PK-profile. Due to the lack of data in human plasma and VH, it is difficult to validate our findings and to draw conclusions on the ability of interspecies translation of this model. A possible reason for the unsuccessful translation may be due to the fact that the patients in the human study are elderly patients with macular oedema. This could lead to a different PK profile than when the study was done in healthy individuals. Although it is possible to integrate disease states in the OCAT™ model by adapting the physiological parameters, it is difficult to implement these changes in the model. This is due to the lack of accurate, quantitative data on how these disease state factors alter ocular PK.

5.2.2 Oral levofloxacin and gatifloxacin

The final output of the ocular levofloxacin model (Fig. 4.7) showed that it was unable to capture the observed levofloxacin levels from the Pea et al. study. This contrasts with the studies used by Le Merdy et al. to validate the model, where it successfully predicted the plasma and AH levofloxacin levels (75). Reasons for this could be the

variation in patient population and the sampling procedure. 101 patients were enrolled in the study of Pea et al., with ages ranging from 45 to 90 years old, body weight ranging from 45 to 110 kg and serum creatinine CL ranging from 0.40 to 1.68 mL/min/kg body weight (80). The sampling procedure was conducted by dividing the 101 patients into 9 groups. Samples from the first group were then collected between 1.5-2 hours after the last oral dose, in the second group after 2-2.5 hours, in the third after 2.5-3 hours, and so on. There is thus variation in the sampling times and the fact that each sample at the different time points is not of the same set of individuals. Although Pea et al. stated that the individuals were randomly assigned to a group, this does not fully diminish the variability in the population and during sampling. Additionally, it was not stated whether the patients were fasted or fed when the oral doses were administered. In the model, the patients were set to a fed state for the second oral dose, as there was a 10-hour interval between the first and second dose in the study. It could be argued that the first dose was given following an overnight fast, and the second dose was administered in a fed state. Furthermore, this change in fed state for the second dose gave better predictions than in the fasted state. Pea et al. observed a linear correlation between the plasma levofloxacin levels and AH levels ($r = 0.81$) (80). However, this observation is not reflected in the ocular model. The predicted levofloxacin AH levels seem to rise gradually over time in a linear fashion, while the plasma concentrations exhibit a significantly different pattern, namely an oral PK profile with an absorption and elimination phase, represented by the upwards and downwards slope.

The observed data for oral gatifloxacin from Rajpal et al. consisted of 3 measurements from 44 individuals with non-inflamed eyes in both plasma and VH. The model underpredicted the gatifloxacin levels in both matrices (see Fig. 4.8). The overall pattern of the plasma predictions seems to have a similar downwards slope as the observed levels, however it is difficult to make conclusions based on only three measurements. Predicted VH levels seem to exhibit a constant level, however, a minuscule increase in concentration can be observed in the predictions.

It should be noted that Le Merdy et al. used an older version of GastroPlus® (version 9.8), while our experiments were conducted using version 9.9 (75). Typically, this would not result in significant differences in the model predictions, but potential changes cannot entirely be ruled out. It is apparent that the topical ocular models were unable to capture the PK profiles of the oral studies. Besides the variability in the patient populations, another reason could be that the model was more adapted to the anterior compartments of the eye than the posterior compartments. Although Le Merdy et al. validated both models for oral administration, they were not able to capture the observed plasma levels

from the studies used in this thesis to validate our predictions. A reason for this could be that the studies used by Le Merdy et al. for validation were both randomized, controlled studies with healthy individuals. Although both models were adapted to mimic the respective study populations in this experiment, the models were not able to capture the observations.

6 CONCLUSION

This thesis offers an extensive overview on what is currently known about the PK processes in the anterior and posterior segment of the eye. This knowledge is crucial for the development of ocular drugs. To date, more is known about topical PK (anterior segment PK) than intravitreal PK (posterior segment PK). Both administration routes are widely used in ophthalmology practice. Certain aspects of intravitreal PK remain unclear in the available literature. Further investigation in these topics could help in developing intravitreal drugs, especially concerning transporters, as these are regarded to play important roles in the PK in the anterior segment.

The knowledge gained from the literature search was applied by conducting two experiments using the OCAT™ model in GastroPlus®. In the available literature, the number of ocular PBPK models available is sparse. The published PBPK studies done with the OCAT™ model in GastroPlus® are from the same authors, Le Merdy et al. PBPK modelling in the eye is therefore relatively new. These experiments provide an initial look at how PBPK modelling integrates ocular PK mechanistically, while also displaying the advantages and limitations of the model. The first experiment with TA attempts to perform interspecies extrapolation from rabbit to human. Although complete validation of the human model is not possible for this experiment, the model offers insights into how the ocular PK of TA might look like in human. The second experiment illustrates that a validated ocular model for a specific dosage form may not accurately predict the observations for the same drug in a different dosage form.

Overall, it is evident that *in vivo* validation is of utmost importance in both ocular PK research and PBPK modelling. The invasiveness of conducting these ocular PK experiments and the significant number of animals required are major factors contributing to the unclarified aspects of ocular PK. This thesis highlights the complexities of ocular PK and the critical need for further investigation and validation of diverse topics. This is essential for the efficient development of more effective and safe ocular drugs.

PBPK modelling is one of the many tools that can further optimize ocular drug development. As future studies on ocular drug delivery are conducted, further optimization of the OCAT™ model is needed to predict ocular drug levels more accurately. With increasing data on ocular PK from future literature, the ability of the OCAT™ model to predict *in vivo* ocular PK will improve, aiding in future ocular drug development.

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8 SUPPLEMENTARY

METHODS

Full search term human PK studies

("Pharmacokinetics"[Mesh] OR "Pharmacokinetic*" OR "Drug Kinetics" OR "PK" OR “Drug disposition”) AND ("ophthalmic*" OR "ocular*" OR “eye”) AND ("aqueous humor concentration*" OR "serum concentration*" OR "plasma concentration*" OR "blood concentration*" OR "vitreous humor concentration*" OR "tear fluid*") AND ("concentration*" OR “level*” OR "drug concentration*" OR "drug level*" OR "drug exposure") AND ("human*") AND ("intravitreal*" OR "topical*" OR "subconjunctival*" OR "oral*" OR "intravenous*" OR “intraocular”)

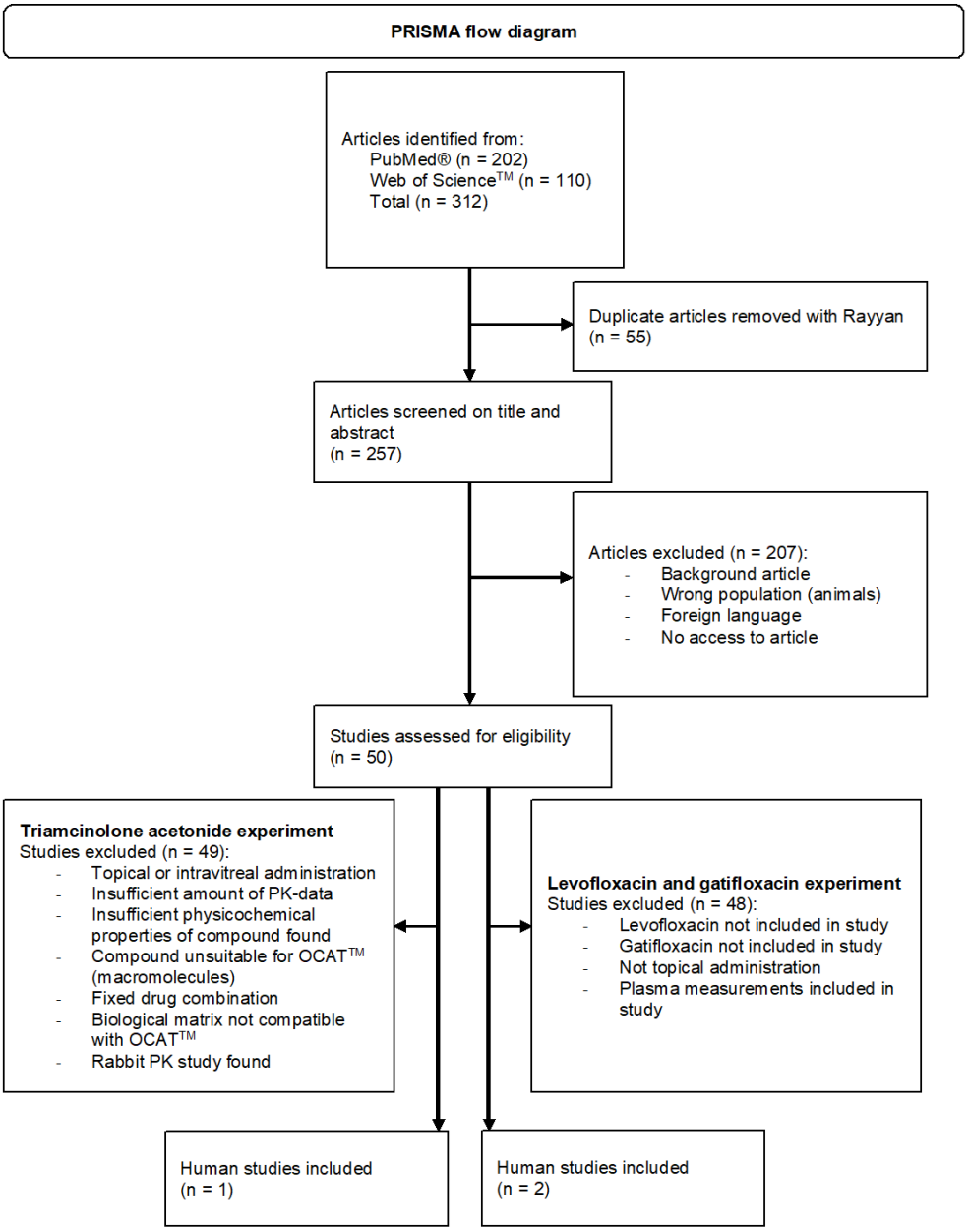

Figure 3.1: PRISMA flow diagram. Template adapted from Reference (161).

Weibull functions

Human TA: $\% \, Dose \, released = 97.397 \, x \left(1 - e^{\frac{-(t^{1.2092})}{529.51}}\right)$

Rabbit TA: $\% \, Dose \, released = 100 \, x \left(1 - e^{\frac{-(t^{1.5582})}{4.195 \, x \, 10^{-4}}}\right)$

Wherein: t: time (h)

Table 3. Adjusted parameter values for the intravitreal TA OCAT™ model. All other parameters were predicted with ADMET or left at their default values in GastroPlus®. MO: manual optimization. Table layout adapted from Reference (75).

Parameter	Definition	Units	Triamcinolone acetonide (rabbit/human)	Source
Compound parameters				
MW	Molecular weight	g/mol	434.5	(162)
Log P	Log octanol/water partition coefficient	-	2.53	(163)
Solubility	Maximum amount dissolved in water	mg/mL	0.02/0.2	(164)
PK parameters				
F_u	Plasma unbound percent	%	32	(165)
CL	Total clearance	L/h/kg	0.57/37	(166)/(167)
V_d	Volume of distribution	L/kg	0.70/103	(166)/(167)
K12	Rate constant for transfer from central to peripheral compartment	h^{-1}	5.3	(166)
K21	Rate constant for transfer from peripheral to central compartment	h^{-1}	3.48	(166)
OCAT™ parameters				
Bmax	Maximum specific binding per mg melanin	nmol/mg melanin	22.43	(168)
Kd	Equilibrium binding constant of drug to melanin	µM	0.024	(168)
$Perm_{VH}$	VH permeability	cm/s	6.2×10^{-9}/6.2×10^{-5}	MO

Master dissertation submitted to the faculty of Pharmaceutical Sciences, performed in collaboration with the Laboratory of Medical Biochemistry and Clinical Analysis

Promotor: Prof. Dr. An Vermeulen

Commissioners: Prof. Dr. Stefaan De Smedt and Prof. Dr. Mathias Devreese

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