A COMPREHENSIVE INSIGHT ON OCULAR PHARMACOKINETICS

Vibhuti Agrahari[1], **Abhirup Mandal**[1], **Vivek Agrahari**[1,2], **Hoang My Trinh**[1], **Mary Joseph**[1], **Animikh Ray**[1], **Hicheme Hadji**[2], **Ranjana Mitra**[1], **Dhananjay Pal**[1], and **Ashim K. Mitra**[1,*]

[1] Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 2464 Charlotte Street, Kansas City, MO 64108, USA

[2] Bayer HealthCare LLC, Shawnee Mission Pkwy, Shawnee, KS 66216, USA

[3] University of Algiers, Faculty of Pharmacy, Algeria

Abstract

Eye is a distinctive organ with protective anatomy and physiology. Several pharmacokinetics compartment model of ocular drug delivery has been developed for describing the absorption, distribution and elimination of ocular drugs in the eye. Determining pharmacokinetics parameters in ocular tissues is a major challenge because of the complex anatomy and dynamic physiological barrier of the eye. In this review, pharmacokinetics of these compartments exploring different drugs, delivery systems and routes of administration are discussed including factors affecting intraocular bioavailability. Factors such as pre-corneal fluid drainage, drug binding to tear proteins, systemic drug absorption, corneal factors, melanin binding, drug metabolism renders ocular delivery challenging and elaborated in this manuscript. Several compartment models are discussed those are developed in ocular drug delivery to study the pharmacokinetics parameters. There are several transporters present in both anterior and posterior segments of the eye which play a significant role in ocular pharmacokinetics and summarized briefly. Moreover, several ocular pharmacokinetics animal models and relevant studies are reviewed and discussed in addition to the pharmacokinetics of various ocular formulations.

Keywords

ocular pharmacokinetics; compartment models; animal models; simulation study; anterior chamber; posterior segment

1. INTRODUCTION

Eye is a sensitive organ and protected from foreign materials by its curved architecture, compartmental organization, impermeable epithelium, tear secretion and ocular drainage

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*Corresponding Author: Ashim K. Mitra, Ph.D., University of Missouri Curators' Professor of Pharmacy, Chair, Division of Pharmaceutical Sciences, Vice-Provost for Interdisciplinary Research, University of Missouri-Kansas City, Phone: 816-235-1615, Fax: 816-235-5779, mitraa@umkc.edu.

11. CONFLICT OF INTEREST

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pathways to clear any foreign object (1-3). Conventional drug delivery systems such as eye drops, suspensions and ointments are frequently indicated for ocular diseases albeit with several disadvantages. Conversely, new approaches such as nano/microparticle, nanosuspension, nano/microemulsion, liposomes, nanomicelles, and dendrimers may overcome the above drawbacks of conventional systems (4). However, most important tools to develop novel ocular delivery systems are to study ocular pharmacokinetics models. In general, the objectives of pharmacokinetics studies involve the study of time and concentration relationships of the administered drugs (5).

2. OCULAR ANATOMY AND PHYSIOLOGY

Eye is comprised of three layers; connective, vascular, and neural tissues. The connective tissue is consists of transparent cornea connected to white sclera through the limbus. The vascular tissue is composed of choroid as well as two ciliary bodies in the middle connected by the iris in front of the globe. The retina constitutes the neural tissue, which has the function of transmitting the electrical impulse to the brain through the optic nerve. There is also an important transparent entity inside the eye called the lens, which is located behind iris and positioned between ciliary bodies by two suspension ligaments known as zonule of zinn (6), (7). The ocular globe consists of an anterior segment (filled with the aqueous humor) and posterior segment (containing vitreous humor). The anterior segment represents a smaller part of the eye which consists of the cornea, conjunctiva, iris-ciliary body (ICB), lens and aqueous humor. In contrast, posterior segment represents the major ocular structure, consisting of sclera, choroid and retina surrounding the vitreous cavity filled with the vitreous humor (Fig.1) (8), (9).

3. BARRIERS OF OCULAR DRUG DELIVERY

Ocular structures possess distinctive anatomical and physiological barriers as illustrated in Fig. 1(10). After topical administration drug is absorbed through either the corneal or non-corneal routes discussed elsewhere (8). Administration of medications via local or systemic routes must overcome these barriers to achieve an effective concentration in retina and vitreous. The blood-ocular barrier (BOB) is responsible for maintaining the fluid composition and aqueous humor as well as controlling the inflow/outflow of aqueous humor thereby maintaining optimum pressure inside the eye. Barriers in ocular drug delivery are classified as physiological and anatomical. Physiological barriers include (i) tear turn over, (ii) naso-lachrymal drainage and (iii) blinking. Anatomical barriers comprise of static and dynamic barriers, which limits drug entry into the anterior segment. Static barrier consists of corneal epithelium, stroma, and blood–aqueous barrier (BAB). While dynamic barriers involved in conjunctival blood and lymph flow as well as tear drainage. BAB comprises of tight junctions of the non-pigmented epithelium of the ciliary body, junctions of the iridial tissues, and the iris blood vessels. These factors together limit drug entry to the anterior chamber of the eye.

In the posterior segment, static barriers include sclera, Bruch's membrane-choroid (BC), retinal pigment epithelium (RPE) and conjunctiva while dynamic barriers involved in drug clearance mechanism through blood and lymphatic vessels. Traditionally, BOB consist of

BAB and Blood-retinal barrier (BRB) which limits the availability of therapeutic agents in ocular compartments includes tight junctions in retinal capillary endothelial cells and RPE cells. BAB restricts molecular movement from blood to aqueous humor through iris ciliary capillaries (4, 11-13). Another barrier in ocular delivery is mucin which is a gel like structure which plays a protective role into eye. Mucin form barrier layers on the corneal and conjunctival surface of the eye. Mucin is also considered as a permeation barrier which restricts the ocular drug absorption but no direct evidence to show that it restricts the bioavailability of topical ocular drugs. Mucin layer may limit the diffusion of the large molecule drugs. The role of mucin barrier in drug absorption is not self-evident (14). In addition, efflux pumps such as P-glycoprotein (P-gp), multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP) expressed on the capillary endothelium represents important barrier to drug absorption (15). Following section describes the factor affecting intraocular bioavailability which renders ocular delivery challenging.

3.1. Factors affecting intraocular bioavailability

Poor drug bioavailability is a major concern associated with ocular dosage forms mainly due to the precorneal loss following topical administration. Because of a number of physiological and anatomical constraints, only a small portion of topically instilled dose can be absorbed. In addition to that several other factors such as solution drainage, lacrimation, tear turnover, tear dilution and conjunctival absorption; the very low permeability of the corneal epithelial membrane play critical role in poor ocular bioavailability of dosage forms. Therefore, to improve ocular permeability, a balance of lipophilicity and hydrophilicity must be achieved (16, 17).

3.1.1. Pre-corneal fluid drainage—Pre-corneal fluid drainage is one of the main reasons for low ocular drug absorption (4, 18). After instillation, a major portion of an instilled volume (approx. 80 to 90%) is drained into the naso-lacrimal duct. Naso-lacrimal drainage helps maintaining the volume of pre-corneal fluid about 7 to 10 μl at any time (19). A natural protective physiological mechanism causes loss of any excess fluid present; it's drained out through the naso-lacrimal duct. Factors influencing the drainage rate are: (a) Instilled volume: Higher the instilled volume more will be the rate of solution drainage from the conjunctival sac; (b) Viscosity: Increasing viscosity of an instilled dose can extend the residence time of solution in the conjunctival sac; (c) pH: The physiological pH of tear fluid is 7.4. Instillation of acidic or alkaline solution results in excessive tear secretion and loss of drug. The ophthalmic preparations are usually pH adjusted in the range of 7.0 to 7.7; (d) Tonicity and drug type: The drug can also alter the normal ocular physiological process for example; epinephrine can induce tear production, while local anesthetics such as tetracaine can suppress it. Ophthalmic formulation for topical delivery should be isotonic with tear. The increased tonicity of the topically applied formulation can be immediately diluted by the tears. Also, tears can adjust the tonicity by osmosis. The tonicity of ophthalmic solution is adjusted to physiologic values by using sodium chloride or mannitol (20).

3.1.2. Drug binding to tear proteins—Tear fluid contains approximately 0.7% of total body protein. Drug binding to these tear proteins may result in a reduction in concentration of total available free drug for required pharmacological action at the target site (8).

3.1.3. Systemic drug absorption—Systemic absorption may take place either directly from the conjunctival sac or after the solution flow to the nasal cavity. A large portion of the topically applied dose may be absorbed systematically (Fig. 2) through the naso-lacrimal duct drainage, which may lead to potential systemic side effects. Systemic absorption of drug through conjunctiva is considered to be nonproductive due to the presence of conjunctival blood capillaries and lymphatics. It can cause significant drug loss into the systemic circulation thereby lowering ocular bioavailability (8).

3.1.4. Corneal Factors—Cornea is made of six different layers (including a novel pre-descemet's layer known as Dua's layer) (21). The epithelial layer of the cornea is lipoidal in nature while stroma is hydrophilic in nature which comprises 90% of the corneal thickness. Endothelium is the innermost layer separating barrier between the stroma and aqueous humor. This layer helps to maintain the aqueous humor and corneal transparency due to its selective carrier-mediated transport and secretory function. (22).

3.1.5. Melanin binding—The melanin pigment present in the iris and ciliary body may also change the ocular bioavailability of a topically administered drug (8). Drugs such as ephedrine and timolol have a high binding capacity for melanin, and only a very small portion of the bound drug can release at a very slow rate (23).

3.1.6. Drug metabolism—Many enzymes (cytochrome P450, aldehyde oxidase, aldo/ketone reductase, cyclooxygenase, monoamine oxidase, hydrolase, and transferase) are expressed in ocular tissues such as cornea, lens, iris-ciliary body and retina. These enzymes can metabolize the active drug, leading to decrease in ocular drug bioavailability (24, 25).

4. ROUTES OF ADMINISTRATION

The conventional routes for ocular drug delivery include topical as well as parenteral administration. However, these routes face several challenges that limit their success in achieving the desired drug concentrations at the target tissue. To overcome these barriers several alternative routes of drug administration are proposed.

4.1. Intravitreal injection (IVT)

IVT delivers therapeutic system such as solution, suspension, or depot formulation directly injected into the vitreous humor through pars plana. After achieving equilibrium within vitreous, drug elimination can proceed either through the retina or the anterior chamber through aqueous humor. IVT is a highly efficient method for delivering drugs into the posterior segment. However, repetitive dosing may damage eye structures leading to retinal detachment, cataract, hyperemia, and endophthalmitis (26).

4.2. Subconjunctival route (SC)

SC delivers the dose beneath the conjunctival membrane that lines the inner surface of the eyelid, circumventing the cornea and conjunctiva. It is considered less invasive compared to intravitreal injections. Moreover, SC administration allows the dose to pass through the sclera and enter the posterior segment (27).

4.3. Retrobulbar route (RB)

RB represents injection through the eyelid and the orbital fascia placing the dose directly the retrobulbar space. It is a delicate procedure associated with the risk of damaging the optic nerve. However, this route is primarily indicated for the delivery of anesthetic agents which can cause changes in the intraocular pressure (28).

4.4. Peribulbar route (PB)

PB involves injections above and/or below the globe. This route is suitable for the delivery of anesthesia in case of cataract surgery. PB shows reduced risk of injury to the intraorbital structure relative to the anesthetic deposited outside the muscle cone (29).

4.5. Sub-Tenon route (ST)

ST injection is administered into a cavity between Tenon's capsule and sclera. This approach requires a blunt cannula for dose administration. Due to lower complications and avoidance of sharp needles, the sub-tenon route is believed to be a better route for delivering anesthesia compared to the retrobulbar and peribulbar routes (29).

4.6. Intracameral route (IC)

IC involves drug delivery to the anterior chamber by injection. It is a cost-effective and efficient method of delivering antibiotics compared with topical antibiotics and antifungal agents. IC route is preferred to prevent the occurrence of endophthalmitis after cataract surgery (30).

5. OCULAR PHARMACOKINETICS

Ocular pharmacokinetics is an assessment of drug absorption in relation to time and dosage of administered drugs. Drug concentrations change in tissues or fluids of the eye when it is administered in various dosage forms through different routes. Ocular tissues barriers pose major challenges in delivering drug at therapeutic concentrations to the desired location [8]. To study pharmacokinetics, fictitious body spaces needs to be considered through which drug molecules traverse, and distribute. These areas include the extracellular and intracellular spaces along with the intravascular compartment. In order to study ocular pharmacokinetics, eye components such as a) tear film and cul-de-sac, b) anterior chamber, c) vitreous cavity and d) retro or periocular space should be considered as compartments. Each eye could be considered as an individual and may be as a multi-competent structure. Ocular pharmacokinetics has frequently performed by a multi-compartment model, assuming a homogeneous distribution of drugs in each ocular tissue. A major drawback of the compartmental model is a lack of detailed information on local distribution in various ocular structures.

5.1. Ocular compartments

In respect to ocular drug delivery, eye can be clinically visualized as compartments. To delineate ocular pharmacokinetics parameters the main focus should be on ocular compartments which include the anterior chamber, vitreous cavity, tear film, cul-de-sac and

finally retro/periocular space. In following subsections, pharmacokinetics of these compartments exploring different drugs and routes of administration are discussed.

5.1.1. Cul-de-sac and tear film compartment—Cul-de-sac is sometimes referred to as the conjunctival sac. It is a narrow pocket where palpebral and bulbar conjunctiva meets in the lower eyelid, with deeper recess in the upper eyelid. It is the space between bottom of the eyelid and globe. Following topical administration the dose comes into contact with the cornea. Absorption of topically administered drug is through permeation across the cornea from pre-corneal tear film or through systemic absorption through local blood capillaries at cul-de-sac (5, 31). Tear film and cul-de-sac may enlarge up to 30μl in volume under enlarged conditions (8, 31). Nonetheless, this expansion is not enough to hold 40 to 70μl of a commercial topical eye formulation. Hence most of the dose is washed out immediately following instillation. As a result, less than 5% of the drug reaches intraocular tissues (8), (32). Topical drugs and their metabolites may be eliminated through various mechanisms. The most common ones are the initial outflow of drug by blinking mechanism of the eyelids and through the naso-lacrimal route. Administered dose in the anterior chamber may be eliminated through aqueous humor outflow (5).

5.1.2. Anterior chamber—Topically administered agents are absorbed through the cornea then transferred into the anterior chamber. The dose is then carried away by aqueous flow and by diffusion into the blood circulation through anterior uvea (8). Drug molecules can permeates through the corneal epithelium by utilizing two pathways i.e. transcellular pathway for lipophilic drugs or paracellular pathways for hydrophilic drugs (33). Lipophilic drugs may be sequestered in epithelial cells from which molecules gradually released to the corneal stroma and further anterior chamber (5). The lipophilic nature of corneal epithelium acts as the rate limiting barrier for hydrophilic drugs (33). Once the drug reaches to the aqueous humor; it can easily be distributed to the iris and the ciliary body. Drug molecules can bind to melanin and create a reservoir, from which the drug is slowly released to the surrounding cells, hence prolonging drug activity (34). Presence of esterase and lysosomal enzymes in the anterior chamber play an important role in the bioreversion of amino acid prodrugs (35). Drug elimination from anterior compartment primarily takes place through aqueous humor by two different mechanisms. First includes the chamber angle and Sclemm's canal and second mechanism involves blood flow in anterior uvea (5). Several drugs are cleared through uveal blood flow (8). In general, lipophilic drugs are more rapidly eliminated relative to hydrophilic drugs.

5.1.3. Vitreous cavity—Drugs administered intravitreally offer direct access to vitreous cavity and retina. Once injected into the vitreous cavity, it may take several hours for drug to diffuse across the entire vitreous humor. Drug permeation from the vitreous cavity to the choroid is slow due to hindrance by RPE whereas drug diffusion from the vitreous to the retina is restricted by the internal limiting membrane (ILM) (5). Several factors control the drug concentration in vitreous cavity. Such factors include initial dose, volume of distribution and the rate of elimination (36, 37). In the vitreous cavity drug can be eliminated through anterior and/or posterior routes(5). Elimination of drug can be influenced primarily by two factors: volume of distribution and elimination half-life (5, 37).

5.1.4. Retro/periocular space—Periocular refers to periphery or the region surrounding eyeball, within the orbit. Drugs administered through a periocular route can be considered as an effective route for drug delivery to the posterior eye segment. Drugs administered through this route can reach the posterior segment through several pathways such as transscleral pathway, systemic circulation and choroidal blood flow. The anterior pathway defines tear film, cornea, aqueous humor and vitreous humor (8, 13). Since periocular space is the region surrounding eye, it can produce similar pharmacokinetics within the anterior or vitreous compartments, which have already been discussed earlier.

5.2. Pharmacokinetics compartment models in ocular drug delivery

Several pharmacokinetic compartment model of ocular drug delivery has been developed for describing the absorption, distribution and elimination of ocular drugs in the eye. Components of the eye such as tear film, cornea, aqueous humor, lens and vitreous humor are transparent. These components have no direct blood supply (38). The blood supply to the eye is complex: it is supplied by the ophthalmic artery and choroidal plexus. Choroidal blood vessels allow easy drug distribution from the bloodstream to the extravascular choroid (39). However, access of choroidal drug into the retina is limited by the RPE barrier. Systemic drugs circulate through iris, ciliary body, choroid, and retina. Though, blood vessel walls in the iris and retina have tight junctions between the endothelial cells that slow drug permeation across the vessel walls (40).

To study compartment models, each component of the eye can be considered as the distinct compartment separated by a barrier from the other compartment. Therefore, flow between adjacent compartments takes more time than diffusion. For example, considering tear as one compartment (Fig. 3) with constant turnover than inflow of the lachrymal fluid is constant and equal to the outflow through the puncta. Once hydrophilic drug instilled it mixed rapidly and tear flow carries away a portion per unit time dependent on the drug concentration present (38). Similarly cornea can also be considered as one compartment. This is expressed by the following equation 1.

$$\frac{dC_d}{dt} = k_0 C_d t \quad \text{Eq. 1}$$

Where, C_d = the concentration of drug in tears at time t;

dC_d/dt = the change in concertation C_d during an interval time;

k_0 = a proportionality constant of drug loss to the nasolacrimal duct dependent on the flow rate and volume of the tear compartment

It is expected that 99% of the drug is lost from the precorneal area. The maximum amount of the drug is absorbed into the systemic circulation via the conjunctival membrane and the naso-lacrimal drainage system (Fig. 3). Once the drug is in the blood circulation, it distributed to other parts of the body which is considered as the peripheral compartment, followed by metabolism and excretion. Elimination routes of the eye also affect the local tissue concentration. Therefore, the concentration in the aqueous chamber and the vitreous body is not homogeneous but distribute complicatedly according to the elimination rate

across the surrounding tissues. However, if 1 % of instilled amount penetrates the corneal epithelial barrier and enters the stroma then cornea is considered as second compartment (Fig. 4). According to the two compartment model, drug is diffuses across the barrier from low concentration to high concentration (38) based on the Fick's first law of diffusion (equation 2).

$$\frac{dC_d}{dt} = k_{dc} A (C_d - C_y) \quad \text{Eq. 2}$$

Where, dC_d/dt = the net drug moving from tears to cornea per unit of time;

A = the area of the tear-corneal interface;

C_d = concentration of drug in tears

C_y = concentration of drug in cornea; and

K_{dc} = the permeability constant from tears to the cornea.

The permeability constant in the reverse direction would be k_{cd}.

As soon as the concentration in the two compartments i.e. tears and the cornea reached to the equilibrium, drug can no longer penetrate inward. Similarly, diffusion of the drug from the cornea to aqueous humor is in the same way as of tears to cornea (38). To complete the model, the elimination transfer coefficient from the aqueous to the blood plasma is given by equation 3.

$$\frac{dC_4}{dt} = k_{dc} A (C_d - C_y) \quad \text{Eq. 3}$$

Where, dC_d/dt = the amount of drug penetrating the barrier during a unit of time.

A = the area of the tear-corneal interface;

C_c = concentration of drug in cornea;

C_d = concentration of drug in aqueous; and

K_{dc} = the permeability constant from cornea to aqueous.

The more complex pharmacokinetics model is based on Fick's second law of diffusion which assumes a spherical modified cylindrical eye for ocular delivery. This model showed the three pathways for drug transport across the surface of the eye. These pathways are anterior aqueous chamber, the posterior aqueous chamber and the retina/choroids/scleral membrane covering the vitreous body (41). The three-compartment pharmacokinetics model has been demonstrated to determine the pharmacokinetics of various peptide drugs such as insulin, glucagon, luteinizing hormone-releasing hormone (LHRH) and leu-enkephalin through ocular routes (42). The major compartments are the precorneal area, cornea, and aqueous humor Fig. 5.

Makoid and Robinson (43) used a four-compartment model (Fig. 6) following the topical administration of pilocarpine to the Albino rabbit eye. The results demonstrated that cornea1 epithelium is a reservoir for pilocarpine and act as a barrier to drug penetration whereas; the cornea1 stroma and endothelium are kinetically homogeneous with aqueous humor.

Figure 7 depicts five compartment model consists of precorneal area, cornea, aqueous humor, iris-ciliary body and lens. It is assumed that drug movement between compartments is a reversible process. The drug elimination from the eye, was assumed to occur only from the aqueous humor and iris-ciliary body(1).

5.3 Pharmacokinetic simulation model

The permeability trend of drugs and structure of BOB have been known for decades. However, no reports available for the distribution of drug from blood to the eye. Any model for ocular drug distribution would be a beneficial tool in drug discovery. The simulation model (Fig. 8) could be a significant approach to estimate the delivery of ocular drugs. Study proposed by Urtii ei al, predicted the ocular distribution of systemic drugs by using a simulation model. The distribution clearance between vitreous and plasma was obtained from a Quantitative Structure Property Relationship (QSPR) model for clearance of intravitreal drugs (37). The reliable predictions were obtained using computational value of ocular distribution clearance, systemic concentrations of unbound drug, and a simple pharmacokinetics model. In summary, a pharmacokinetics simulation model has been built for prediction of drug concentrations in the vitreous. It is based on the unbound drug concentrations in the plasma and the computational estimate for the distribution clearance between blood circulation and the eye. This model could accurately predict the drug distribution to the eye (40).

The requirement of the simulation study is that the drug concentrations of plasma and vitreous must be available. Therefore, based on the literature, rabbit simulations were performed for 10 compounds and human simulations with one drug. The simulations matched the experimental data well as showed in figure 9. A pharmacokinetic model based on computational distribution clearance and free drug concentrations in plasma was capable of predicting the drug concentrations in vitreous. The vitreal concentrations of the studied compounds spanned a 1000-fold range of concentrations, and yet the simulated drug concentrations were relatively close to the experimental values in each case. The simulations with computational and experimental values of distribution clearance showed similar predictability. (40).

5.4 Pharmacokinetics animal models

Determine the pharmacokinetic parameters in ocular tissues are a major challenge because of the complex anatomy and dynamic physiological barrier of the eye. In drug development process, human pharmacokinetics is generally assessed after per os or intravenous administration by sampling plasma at different time intervals. However, for drugs administered through ocular route with a local therapeutic effect, cannot be sampled. A very few exceptions are made such as monitoring of drug levels in biopsies or aqueous humor collected from patients subjected to ophthalmic surgical procedures (19). Therefore, animal

models are used to study the drugs distribution in ocular tissues. The ocular characteristics of animal models permit to extrapolate to human pharmacokinetics.

Several pharmacokinetics animal models have been developed to study the distribution and elimination of ocular drugs for different routes of administration (41). The rabbit is a commonly used species for preclinical ocular PK studies. The pharmacokinetics parameters in the rabbit have shown the predictable parameters in human (44, 45). Even though the human eye has a higher retina vascular and larger vitreous cavity, smaller lens and larger serum compartment than rabbits (46). These both share common characteristics (1). These factors may contribute slight difference in pharmacokinetics of the drug in human and rabbit. Still, rabbit model is established to study the pharmacokinetic parameters during ocular drug development (37).

Ozcimen et al. used rabbit uveitis model to study ocular pharmacokinetics of intravenously administered tigecycline in various compartments. Significant low therapeutic levels were observed in vitreous and aqueous humor while a higher concentration was observed in plasma (47). This result suggests that intravenously administration of tigecycline is not suitable route for the treatment of bacterial endophthalmitis.

In another study, Dutch-belted rabbits were used to compare the pharmacokinetics of intravitreal bevacizumab and ranibizumab (46). The half-life of bevacizumab was 4.32 days and ranibizumab was 2.88 days in rabbit, maximum concentration in the vitreous cavity was 400 μg/mL at day 1 and ranibizumab was 162 μg/ml. Because bevacizumab (149 KD) has larger molecule size than ranibizumab (48 KD), ranibizumab could have higher retina penetration and faster elimination. In another study, Xu L. et al., have reported that the one compartment model is the best fit model of the pharmacokinetics of ranibizumab in 674 patients with age-related macular degeneration (48). It was observed that ranibizumab is eliminated from systemic circulation by the first order elimination. The elimination half-life in the vitreous was 9 days and intrinsic systemic elimination half-life is 2 hours.

Compared to rabbit model, pharmacokinetics of ranibizumab is slightly different in monkey animal model. Gaudreault J et al have reported that half-life of 0.5 mg ranibizumab was 2.6 days compare to 2.88 days in rabbit and vitreous cavity maximum concentration was 169 μg/ml at 6 hours compare to 162 μg/ml at day 1 in rabbit study (46, 49). Proksch et al also studied the ocular pharmacokinetics for mapracorat, a selective glucocorticoid receptor agonist, in rabbit and monkey (50). Mapracorat was administered single or repeated with a dose range from 0.01 to 3000 μg/eyes for rabbit and 50 to 3000 μg/eyes for monkey. In both species mapracorat concentration was distributed higher in cornea, conjunctiva than aqueous humor and iris/ciliary body. Those differences are acceptable to have some idea to predict about the pharmacokinetic profile of any drugs. Le et al has also investigated the lampalizumab elimination following the intravitreal injection (51). The slow ocular elimination was observed compared to systemic elimination. Moreover, Drolet DW et al. have shown the pharmacokinetic and safety profile of pegatanib in rhesus monkeys to support the human clinical trial and (52, 53). It has also predicted the human plasma clearance and vitreous humor concentration of pegaptanib (54).

Pharmacokinetic/pharmacodynamics (PK/PD) model of Factor D inhibition in monkeys by lampalizumab for the treatment of geographic atrophy was developed by Le et al. In addition, intravitreal administration of lampalizumab showed therapeutic levels at the target site while minimizing systemic drug exposure to patient (51). Intravitreal injection of lampalizumab resulted in slow ocular elimination compared to systemic elimination (51). These results may be helpful in predicting human PK/PD for lampalizumab.

Despite the fact that rat models have posed various challenges in ocular pharmacokinetic, several studies have shown to be valuable in determining different parameters that are important in establishing PK/PD relationships. Liu et al studied subconjunctival biodegradable microfilms for sustained drug delivery to the anterior segment of rats. Microfilm was composed of prednisolone loaded poly (lactide-co-ε-caprolactone) drug delivery system which was subconjunctivally implanted into rat eyes. These films were able to deliver the drug for 3 months at a rate of 0.002mg/day (55). In addition, Tommaso et al. investigated the effect of micelle loaded with Cyclosporin A (CsA) for topical delivery on rat model for prevention of corneal graft rejection after keratoplasty procedure. Results revealed that CsA-micelle formulation was able to penetrate all corneal layers, a 73% success in cornea graft transplant, 50 % reduction in neovascularization and significant lower edema *in vivo* (56).

Pharmacokinetics of topically administered Ciprofloxacin (0.3 %) through cul-de-sac in equine with no ophthalmic diseases was studied by Hendrix et al. (57). Tears were sampled from cul-de-sac at different time points post dosage and analyzed for the concentration of ciprofloxacin. Ciprofloxacin levels remained above minimum inhibitory concentration necessary to inhibit the growth of 90% of organisms for 6 hours post administration (57). These results may be simulated in human as pharmacokinetics of ciprofloxacin in normal horses is comparable to that of rabbit and human.

Ward et al. investigated the ocular PK properties of besifloxacin in rabbits, monkeys, and humans, using a robust PK study design, and to compare the resulting PK parameters (Cmax and AUC) against the prevalent pathogens isolated from patients with bacterial conjunctivitis. For this study, Dutch-belted and New Zealand composite rabbits and cynomolgus monkeys were used. The male Dutch-belted rabbits received a 50-µL instillation of besifloxacin ophthalmic suspension (0.6%) into the conjunctival sac of each eye as a single bolus dose. At predetermined time intervals after dosing, rabbits were euthanized and the eyes enucleated, frozen, and dissected, with tissues collected separately for each eye. Cynomolgus monkeys (males and females) were used to investigate the ocular and systemic PK following topical ocular administration of besifloxacin ophthalmic suspension (0.6%) into the conjunctival sac of each eye as a single bolus dose. To assess systemic PK, serial blood samples (~0.5 mL) were collected from a femoral vein from one cohort of three monkeys at timed intervals from 5 min to 24 h following topical ocular dosing. After a specified collection times, monkeys were euthanized and eyes were enucleated and snap-frozen for further analysis. For human study sixty-four healthy male or female volunteers were enrolled open-label study and besifloxacin was assessed in human tear fluid. Overall, the ocular PK profile of besifloxacin following topical ocular administration was similar in rabbits and monkeys. The results of this investigation indicated that following topical dosing

of besifloxacin demonstrates good ocular penetration in rabbits and monkeys. Similarly, achieves sustained levels in tears to humans. Slightly, lower maximal besifloxacin levels were observed in humans compared with rabbits or monkeys (Fig 10). This difference is partly due to the blink rate and tear turnover rate affects the dynamics of drug retention on the surface of the eye (58).

Animal models with damaged BRB led to higher drug elimination rate compared to normal animals. Also a higher intravitreal clearance was observed in diseased animals. These results suggested that PK/PD outcome depends on various factors such as drug properties as well as normal or disease model (59). Therefore, appropriate design and selection of animal model to investigate ocular PK/PD is pivotal. Moreover, suitable formulation to overcome these barriers is the key to advance ocular drug delivery.

6. PHARMACOKINETICS OF VARIOUS OPHTHALMIC FORMULATIONS

Pharmacokinetics of drug molecules in ocular tissues including vitreous is non-uniform. There are several factors affecting the ADME of a drug molecule in the eye. While small molecules can rapidly distribute through the vitreous, diffusion of large molecules is highly restricted. The distribution of drug molecules also depends on the molecular weight and physicochemical properties such as structure, solubility, logP, stability of the administered drug. Presence of surface charge also greatly influences the distribution of a drug molecule across ocular tissues. For instance, cationic lipid, polymers can interact with the negatively charged hyaluronan present in the vitreous leading to aggregation and ultimately immobilization inside the ocular tissues. Likewise, nanoparticles with surface charge exhibit differential activities. Polystyrene nanospheres have shown to interact with collagen fibrillar structures present in the sclera, resulting in limited diffusion across the vitreous. In fact, nanoparticles with a zeta potential of −33.3 mV have been reported to diffuse freely across the vitreous in comparison to cationic particles with zeta potential of 11.7 mV. Such complexities have led to development of several modification techniques such as surface modification of nanoparticles with PEG chains, masking of reactive surface of nanoparticles, targeting specific transporters or receptors present on the cell surface.

6.1. Topical formulations

6.1.1. Suspension, emulsion and solution

6.1.1.1. Restasis®, 0.05% cyclosporine (CsA): CsA a potent immune-modulator is widely indicated in the treatment of ocular surface disorders such as vernal kerato-conjunctivitis, kerato-conjunctivitis sicca (KCS) [dry-eye disease], immune-mediated keratitis, and herpetic stromal keratitis(60). Additionally, systemic administration of CsA at dosages 2 - 15 mg/kg/day can relieve various ocular disorders such as uveitis, Behcet's disease and bird shot retinochoroiditis (61). However, various adverse effects are associated with systemic use including elevated serum creatinine, hypertension and renal dysfunction.

Restasis, an ophthalmic castor oil-water emulsion containing 0.05% CsA was approved by the US FDA in 2002 for the treatment of KCS. Ocular absorption and tissue distribution studies of CsA after topical administration in rats and dogs have shown achievable CsA

concentrations of   0.236 μg/g at the ocular surface and cornea which is considered satisfactory for immunomodulation. However, drug penetration from ocular surface to intraocular tissues was observed to be extremely poor. It is concluded from these studies that drug vehicle retention time is a major determinant of the extent of drug accumulation. This suggests that choice of a vehicle considerably alters drug bioavailability. Tissue accumulation of different combinations of vehicles and CsA concentrations after topical administration has been summarized in Table 1 (62). Results from phase II clinical trials have shown 0.05% CsA to be most consistent in improving patient's symptom. Although, treatment related adverse effects were observed in 22% of the patients in phase III studies indicating the necessity of safer formulations in future (60).

6.1.1.2. Indomethacin ophthalmic suspension: Indom® and

Indocollirio®: Indomethacin, one of the most commonly used non-steroidal anti-inflammatory drug (NSAIDs), is employed for maintaining mydriasis during cataract surgery and reduction of discomfort after refractive surgery or in allergic conjunctivitis. Several studies have shown NSAIDs to be highly beneficial in diabetic retinopathy and age-related macular degeneration. Bucolo et al. has compared the ocular pharmacokinetics of two different topical formulations of indomethacin those are available in the European market. Indom® (Alfa-Intes; ophthalmic suspension containing 0.5% indomethacin and hydroxypropylmethylcellulose; IND-HPMC) and Indocollirio® (Bausch & Lomb; ophthalmic solution containing 0.1% indomethacin and hydroxypropyl-b-cyclodextrin; IND-CD) are the two formulations included in this study. Following single instillation of IND-HPMC and IND-CD in rabbit's eye, peak indomethacin concentrations in aqueous and vitreous were observed to be T_{max} (30min) and T_{max} (60min) respectively. Significantly higher levels of indomethacin with IND-HPMC (AUC_{0-240}, 272.9ng/g /min) were observed in retina in contrast to the IND-CD formulation (AUC_{0-240} 73.5 ng/g/min). However, retinal T_{max} for IND-CD (120min) was slightly higher than IND-HPMC (30min) treated groups. Additionally, drug levels in both aqueous and vitreous were statistically higher with INC-HPMC treated groups in comparison to IND-CD groups (AUC_{0-240} 2039 ng/ml per min vs AUC_{0-240} 427.3 ng/ml per min, AUC_{0-240} 53.8 ng/ml per min vs. AUC_{0-240} 12.5 ng/ml per min, respectively). Higher indomethacin levels in various ocular tissues after topical administration of IND-HPMC in comparison to IND-CD (retina: C_{max} 73.7 ± 6.4 ng/g vs. 25.5 ± 1.73 ng/g; aqueous: C_{max} 952 ± 6.8 ng/ml vs. 163 ±4.1 ng/ml; vitreous C_{max} 31 ± 3.5 ng/ml vs. 6.37 ± 3.6 ng/ml) (P<0.05) indicated the usefulness of HPMC in treating various ocular disorders. Although cyclodextrins have been widely used for increasing the solubility of poorly water soluble drugs, the activity of this compound in ocular tissues is controversial. On the contrary, the bio adhesive nature of HPMC is highly beneficial in improving ocular drug bioavailability (63).

6.1.1.3. Azithromycin (1%) ophthalmic solution: AzaSite®: Azithromycin, a macrolide
antibiotic has shown greater potency against Haemophilus influenza and Neisseria gonorrhea than erythromycin. It is highly effective against the intracellular Chlamydia trachomatis which is responsible for causing infectious blindness in human. Akpek et al. has utilized a polycarbophil-based mucoadhesive formulation to enhance ocular distribution of azithromycin. It was observed that after topical administration in rabbits, drug levels in the

tear film, cornea, conjunctiva and aqueous humor remained above detectable levels even after 6 days. However, the concentrations found in aqueous humor were considerably low in comparisons to other tissues. Polycarbophil formulation resulted in higher bioavailability of azithromycin in various ocular tissues (19.4-fold in tear film, 9.3-fold in conjunctiva, and 4.3-fold in the cornea). The C_{max} and AUC_{0-t} were significantly enhanced following treatment with the formulation. Following 7 day multiple administration regimen, significant increase in $AUC_{(144-288h)}$ (4.2-fold in conjunctiva, 19.6-fold in cornea, and 13.3-fold in aqueous humor) was observed in contrast to the $AUC_{(0-144\ h)}$ after single administration. Table 2 summarizes various pharmacokinetic parameters with this formulation. Higher residence time of azithromycin in ocular tissues may be attributed to the mucoadhesive properties of polycarbophil rendering oral azithromycin single administration highly effective for the treatment of trachoma, which mainly affects the conjunctiva and lids.

Recently, pharmacokinetic properties of a 1.5% azithromycin ophthalmic preparation in medium chain triglyceride oil formulation was studied in rabbits(64). A similar distribution pattern was observed after single and multiple administrations as shown in the previous studies. However, the maximal concentrations of azithromycin achieved in all ocular tissues were significantly lower in comparison to the polycarbophil-based formulation (Table 2) (65).

6.1.2. Nanoparticles

6.1.2.1. δ-cyclodextrin nanoparticle dexamethasone (DexNP) and dorzolamide (DorzNP) eye drops: Johannesson et al. developed δ-cyclodextrin nanoparticle dexamethasone (DexNP) and dorzolamide (DorzNP) eye drops. These formulations were expected to provide sustained high drug concentrations on the eye surface. It was reported that dexamethosone peak concentration (ug/mL ± standard deviation) from DexNP eye drops (636 ± 399.1) was 19-fold higher relative to topical ophthalmic steroid suspension of dexamethasone 0.1% Maxidex® (39.3 ± 18.9) (p < 0.001). After 4 hr, DexNP was still 10 times higher than Maxidex®. Additionally, DexNP resulted in 30-fold higher concentration of dexamethasone in tear fluid over extended time period allowing higher drug amounts to partition into various ocular tissues. Dorzolamide concentration from DorzNP (59.5 ± 76.9) was about 50% higher relative to Trusopt® (40.0 ± 76.7) (p < 0.05) (66).

6.1.2.2. Loteprednoletabonate 0.4% mucus-penetrating nanoparticles: 3- to 40-μm layer of mucus on cornea and conjunctiva presents a unique challenge for topically applied therapeutic agents. A possibility of reduced risk of intraocular pressure elevation for loteprednoletabonate (LE) ophthalmic solution (0.5%), has been compared to other corticosteroids.LE exhibited similar efficacy as compared to other corticosteroids such asprednisolone acetate. However, IOP was reduced to greater extent, thereby presenting its improved profile over other corticosteroids (67). Schopf et al. has conducted a study to improve drug penetration into ocular tissues underlying the mucous barrier with a novel mucus penetrating particle (MPP) technology. Studies were conducted in comparison to Lotemax® (loteprednoletabonate ophthalmic suspension, 0.5%) which has shown relatively higher distribution in corneal tissues, with reduced penetration into the aqueous humor and iris/ciliary body. Topical administration of both formulations (LE-MPP 0.4% and Lotemax

0.5%) in 48 rabbits (96 eyes) resulted in a T_{max} of 0.5h. Additionally, LE-MPP 0.4% exhibited 3-fold higher C_{max} and 2-fold higher AUC0-12h in contrast to Lotemax 0.5% in aqueous humor. Similar patterns were exhibited by LE-MPP 0.4% in the cornea (3.6-fold C_{max} and 1.5-fold AUC_{0-12h}), conjunctiva (2.6-fold C_{max}) and other ocular tissues. These results (Fig. 11) indicate that application of such delivery systems are feasible in order to enhance drug concentration in ocular tissues as well as to reduce dosing frequency for the treatment of various complications (68)

6.1.3. Microspheres

6.1.3.1. Mucoadhesive polymer based ocular microspheres of ganciclovir: Recently ganciclovir chitosan microspheres (GCM) were prepared and evaluated in Wistar rats (69). *In vitro* release study exhibited initial burst (nearly 50 %) in first few minutes. The release rate followed Fickian (R2 = 0.9234, n-value = 0.2329) type of release mechanism. Aqueous humor pharmacokinetic studies revealed a 4.99-fold increase in AUC after topical installation of GCM in comparison of ganciclovir solution. Almost 1.8-fold increase in absorption rate (Ka) of GCM (0.7252h), was observedrelative to ganciclovir solution (1.2981 h). C_{max} of GCM was 2.69-fold higher than ganciclovir group (p < 0.0001) and the delay in T_{max} inferred the sustained release of GCM. It was concluded that the incorporation of ganciclovir into microspheres significantly enhanced relative ocular bioavailability (70).

6.2. Intravitreal injectable formulations

To achieve therapeutic drug concentration to the back of the eye by topical eye drops is very challenging. An invasive method such as intravitreal injection has been exploited to overcome barriers and deliver therapeutics in the retina. Several intravitreal products are commercially available such as Avastin® (Genentech), Lucentis® (Genentech), Macugen® (Eyetech Inc.), and Triesence® (Alcon).

6.2.1. Antibody

6.2.1.1. Avastin® (bevacizumab): Avastin is a recombinant humanized monoclonal IgG1 antibody that inhibits the vascular endothelial growth factor (VEGF). It is indicated for VEGF-mediated diseases such as choroidal neovascularization, central retinal vein occlusion and proliferative diabetic retinopathy. Bakri et al. have reported pharmacokinetics of bevacizumab after intravitreal administration in twenty dutch-belted male rabbits (Table 3). One eye of each of twenty rabbits was injected 1.25 mg of bevacizumab intravitreally. Both eyes of each of 4 rabbits were enucleated at days 1, 3, 8, 15, and 29. Bevacizumab concentrations were measured in aqueous fluid, whole vitreous, and serum. Although concentrations of bevacizumab declined in vitreous humor in a mono exponential manner with a half-life of 4.32 days, concentrations of >10ug/ml were maintained for around 30days. Aqueous humor bevacizumab concentrations reached its peak (37.7ug/ml) in 3 days after intravitreal injection. Elimination half-lives of bevacizumab were found to be 4.88 and 6.86 days in aqueous humor and serum respectively. Fig. 12 demonstrates compartmental model of bevacizumab distribution after intravitreal injection. The model illustrates the distribution of bevacizumab into the aqueous humor and the serum after injection into the vitreous cavity (71).

6.2.2. Aptamer

6.2.2.1. Macugen®: (Pegaptanib) is an aptamer with a molecular weight approximately 50 kDa. Pegaptanib binds and inhibit VEGF. Pharmacokinetic studies on animals (rabbit and monkey) revealed prominent results. Intravitreal injection of pegaptanib sodium in animals demonstrated slow absorption (rate limiting step) into systemic circulation. This rate limiting step may be similar in human. After dosing 3 mg of pegaptanib (10 times recommended dose) the average apparent plasma half-life was 10 days (72). The mean plasma C_{max} of 80 ng/ml appears within 1-4 days in man. The mean area under the curve (AUC) is 25 μg.hr/ml. Absolute bioavailability studies after intravenous administration demonstrated 70-100% in rabbits, dogs and monkeys.

6.2.3. Small molecule drugs

6.2.3.1. Triesence®: Triesence contains triamcinolone acetonide suspension, (40 mg/ml) (73). It is indicated for sympathetic ophthalmia, uveitis, ocular inflammatory conditions and visualization during vitrectomy (72). Pharmacokinetics and ocular tissue distribution studies were conducted on male New Zealand albino rabbits with a dose of 2.1 mg triamcinolone acetonide for intravitreal injection. The drug concentration in retina and choroid was highest and almost seven times higher compared to other ocular tissues. The drug elimination from the ocular tissues was initially rapid followed by a slower elimination phase. Aqueous humor pharmacokinetics of triamcinolone was evaluated in 5 patients following a single IV administration (4 mg) of triamcinolone acetonide. The aqueous humor samples were collected by anterior chamber paracentesis on Days 1 to 31 post injections. Peak aqueous humor concentrations of triamcinolone following single intravitreal administration were found to be 2151 to 7202 ng/mL, half-life 76 to 635 hours, and AUC_{0-t} 231 to 1911 ngh/mL. The mean elimination half-life was 18.7 ± 5.7 days in four non-vitrectomized eyes (4 patients). The elimination half-life of triamcinolone from the vitreous was much faster (3.2 days) in a patient undergone vitrectomy (1 eye) compared to patients without vitrectomy.

6.3. Periocular formulations

6.3.1. Suspension

6.3.1.1. Retaane®: Retaane (anecortave acetate 15 mg) is angiostatic cortisone which inhibits angiogenesis and generally administered once for six months. Augustin et al. conducted a pharmacokinetics study on twenty wet AMD patients treated by two injections of RETAANE® suspension (posterior juxta-scleral depot) over a period of 12 month (74). C_{max} (peak plasma levels) was recorded with observed association of reflux. The results revealed that the reflux was observed in patients who had lower plasma level (4 times lower) of anecortave relative to the patient without reflux. This observation showed the need for a counter pressure device (CPD). Subsequent pharmacokinetic studies were carried out to test the ability of a CPD in eliminating reflux. Results highlighted that reflux was well controlled in all the (100%) patients, with a reflux score of 0 for all eyes. In addition a remarkable observation was there with no loss of dose upon administration using the CPD. A 4-fold rise in C_{max} (mean plasma drug levels) was recorded relative to levels observed in the initial pharmacokinetic study without a CPD (74).

7. PHARMACOKINETICS OF DRUGS UNDER DISEASED CONDITIONS

The corneal stroma is mainly composed of collagen and water which forms a formidable barrier for topically applied hydrophobic drugs (8). Additionally, a large number of diseases are known to alter ocular pharmacokinetics of various drugs and their formulations. Such diseased conditions may precipitate various physiological conditions which may also alter the bioavailability of drug molecules. For instance, patients with fungal keratitis exhibit chronic inflammation at the cornea resulting in poor penetration of the drug in ocular tissues (75). Other ocular infections include uveitis, cytomegalovirus retinitis and proliferative vitreoretinopathy. To overcome such symptoms, drugs are administered in several different ways. In dry-eye patients drugs are administered along with a vehicle/ emulsion to avoid evaporation of limited natural tears. Several ionized drugs are driven into ocular tissues by coulomb-controlled iontophoresis to overcome poor penetration. A few other important diseases that have primarily contributed to the altered ocular pharmacokinetics are retinoblastoma and glaucoma.

7.1. Pharmacokinetics of drugs in glaucoma

Glaucoma is a debilitating disease which leads to blindness in a large population of Americans. It inflicts a huge expense in terms of cost in healthcare and quality of life. Glaucoma causes breakdown of BRB as well as choroidal and retinal neovascularization as the disease progresses. It is necessary to study the pharmacokinetics profile of anti-glaucoma drugs in animal models to determine efficacy in the treatment of glaucoma in patients eventually. Pharmacokinetics parameters of these drugs are different in normal and diseased ocular tissues as illustrated in table 4 (59). In the study performed by Shen et al., (59) it was observed that AUC and C_{max} for both the drugs were significantly lower in the disease model compared to normal animals. This is to be expected as BRB breaks down; there is a decreased exposure of drugs to diseased ocular tissues. This points to need for dose escalation which may lead to dose-related toxicity. Ocular and systemic pharmacokinetics of brimonidine and dexamethasone in animal models with and without blood retina barrier broken-down was studied following single intravitreal injection.

7.2. Pharmacokinetics of drugs in retinoblastoma

Retinoblastoma (Rb) represents cancer of the retina that occurs mostly in children under the age of five. It starts in the retina and spreads through the optic nerve. Carboplatin is mostly used for treatment of Rb. Hence, pharmacokinetics parameters of these drugs should be determined in order to assess efficacy and determine dose. Hayden et al. (76) reported that no toxicity was observed in histopathology sections of rabbit eyes after six consecutive subconjunctival injections of carboplatin at a dose of 5.0 mg. Peak concentration of carboplatin in retina was greater (53.3 ng/mg) in ocular tissues and optic nerve relative to intravitreal injection (21.7 ng/mg) which is the preferred route. Similar results were observed for choroid and optic nerve. Another drug that is being investigated for treatment of Rb is digoxin. As reported by Winter et al. (77), following a dose of 10 µg of digoxin, C_{max} in vitreous is calculated to be 8.5 µg/ml. Hence the drug is expected to be within the therapeutic window for 24 hours following intravitreous administration. It was also observed that digoxin concentration in plasma was significantly below the lower limit of the

therapeutic window following a single dose. Digoxin has a significantly high volume of distribution. This is due to its binding with skeletal and cardiac muscles and kidney. Winter et al. have also reported no measurable concentration of digoxin in the kidney and heart in rabbits. However, this dose was found to be toxic to retina in rabbits. Thus it may not be translated in human in its present form. Therefore, there is an urgent need for development of targeted drug delivery systems.

8. ROLE OF TRANSPORTER IN OCULAR PHARMACOKINETICS

Ocular drug delivery remains a challenge due its distinctive anatomical structure, physiology, several barriers (static and dynamic) and efflux transporters (P-gp, MRP and BCRP). Conversely, there are several xenobiotic transporters present in both anterior and posterior segments of the eye which plays a significant role in ocular pharmacokinetics. While efflux transporters possess barrier for drug permeation, influx transporters serve as drug carrier and can facilitate the absorption and distribution. It is expected that these transporters play a significant role in determining drug exposure to ocular tissue. Though clinical relevance of these ocular transporters have not been fully established, several influx transporters have been exploited to enhance drug bioavailability using transporter-targeted prodrug modifications (Table 5) (8). Also, several ocular drugs including antibiotics, antifungal agents, anti-inflammatory agents, anti-fibrotic agents, anti-glaucoma drugs, H1-receptor antagonists and immune-modulators have been reported to interact with various transporters (78). Efflux transporters are located both on the cornea and retina. These efflux transporters protect the tissue from toxic exposure by excreting the drug molecules after their diffusion inside the cytoplasm. Consequently, the concentrations of some drug molecules in the cell/tissue acquire very less. Dey et al., demonstrated the effect of erythromycin pharmacokinetics in the rabbit and human cornea. The corneal AUC of erythromycin was enhanced in the presence of testosterone- a P-gp inhibitor indicating the influence of P-gp on corneal drug kinetics. Pharmacokinetics of erythromycin was also evaluated in rabbits in the presence of steroids and MK572-a specific inhibitor for MRP. A significant elevation of $AUC_{0-\infty}$, C_{max}, and three-fold differences in rate constant (K_a) of erythromycin with MK571 was observed in corneal absorption indicating the role of MRP. Similarly, significantly enhanced aqueous humor concentrations (K_a, $AUC_{0-\infty}$, and C_{max}) of erythromycin in the presence of cyclosporine A, a P-gp inhibitor was found suggesting P-gp involvement in modulating drug permeation across cornea (79). Similarly pharmacokinetics parameter of erythromycin (K_a, $AUC_{0-\infty}$, C_{max} and C_{last}) was significantly elevated in the presence of steroids (prednisolone, methyl prednisolone and prednisone). Co-administration of methyl prednisolone (MLP) with erythromycin resulted maximum corneal absorption of ^{14}C-erythromycin compared to single specific efflux transporter inhibitor indicating MLP can inhibit both P-gp and MRP (80). In another study, ocular hypotensive prostaglandin (PGF2α) analogs-bimatoprost, latanoprost and travoprost and their free acid forms were found to interact with corneal efflux transporters (P-gp and MRP) and alter drug kinetics. This study indicated only bimatoprost among other PGF2α analogs interacts with P-gp. Dose dependent inhibition of erythromycin efflux in the presence of PGF2α analogs in rPCEC was demonstrated and their K_i values for bimatoprost (82.54 μM), and travoprost (94.77 μM) were similar but significantly higher for latanoprost (163.20 μM). Co-

administration of these PGF2α analogs together or with a steroid also showed higher corneal absorption. Thus, it is conceivable that co-administration of two PGF2α analogs together or with steroids is a viable option to overcome efflux and low bioavailability. Such application may elicit a synergistic pharmacological effect to reduce intraocular pressure (81).

9. CONCLUSION

The application of pharmacokinetics modeling in ocular therapeutics plays a major role in optimizing the ocular therapy for various diseases. The efficacy of ophthalmic formulations not only depends on the drug dose in the formulation, but also, the mechanism of action to maintain the desire effects at the target site. Therefore, pharmacokinetic models are important tools to assess the efficacy of ocular formulations. Hence, these models should be simple, and incorporate all the important pharmacokinetic parameters of the process. However, ambiguity in the estimation of ocular pharmacokinetics parameters remains a major obstacle in the development and delivery of efficient systems to the eye. This can be attributed to the presence of various complex ocular barriers. Also, transporters play an important role in ocular therapeutics (92) and can affect the pharmacokinetics, and efficacy of a drug, but, the clinical relevance of transporters in ocular drug delivery is still unclear. Thus, studies are needed as well to determine the clinical significance of drug transporters in the ocular therapeutics.

Ocular pharmacokinetics studies mostly rely on animal models because of the invasive sampling from the human eye in addition to the ethical issues. Moreover, in the case of human, samples are collected only from the aqueous humor of patients, leaving drug levels in adjacent tissues unknown. Thus, without a precise ocular pharmacokinetic data, it is difficult to predict optimal therapeutic regimens for ophthalmic drugs [21]. Several pharmacokinetics compartment and animal models (rabbit, bovine, pig and monkey) are used to determine the diffusion and partition coefficients of the drug in the ocular tissues. Out of these animal models, rabbit is the most appropriate and remains the species of choice for the assessment and evaluation of ophthalmic products because it is easy to handle and clinically predictable animal model for the evaluation of ocular kinetics. It has been reported that pharmacokinetics parameters such as volume of distribution and clearance differ moderately between rabbit and human. Hence, rabbit to human translation is easy and reliable in ocular pharmacokinetics studies.

Although, studies in animals provide a good overview of the pharmacokinetics but they are resource intense and ethically questionable. The pharmacokinetics parameters obtained from rabbits may not be extrapolated directly to humans, due to certain differences, such as lower corneal thickness, lower tear production, slower blink reflex, higher mucus production, and absence of an uveoscleral outflow pathway in rabbits. Moreover, a sufficient number of intervals must be chosen to adequately characterize absorption, distribution and elimination processes [22]. In addition, a large number of animals are required to obtain statistically significant information's. Thus, alternative approach of ocular pharmacokinetics modeling is required since the current studies are expensive, involving a large number of animals.

In recent years, alternative *ex vivo*, and *in vitro* human and animal cell culture models for ocular investigations are developed. Although, the use of *in vitro* cell models provides cost-benefits and ethical advantages over *in vivo* models, they are still not able to completely mimic the characteristics of the eye. In this regards, the development of 3D *in vitro* culture models that more closely replicates *in vivo*, provide better option. Recently, a novel pharmacokinetics *in vitro* model, the PK-Eye is designed for use during preclinical studies to accelerate the development of longer-lasting therapeutic dosage forms that are needed to treat chronic blinding conditions (93). This low-cost, two compartment *in vitro* model is scaled to the human eye and the model mimics the mass transfer characteristics caused by anterior aqueous outflow. The study showed that the PK-Eye model has many of the features needed to become a practical *in vitro* model with the capacity to contribute to research efforts focused on the development of new ophthalmic medicines.

Ocular microdialysis has the ability to continuously monitor drug concentrations in various tissues and cellular fluids (94). Since, both aqueous and vitreous humors are available in a very low volume this technique can be a powerful tool in ocular pharmacokinetics studies. Ocular microdialysis has been reported for the sampling and analyzing the drug concentrations following topical, systemic and intravitreal administrations. A major limitation of microdialysis technique is that it requires highly sensitive analytical technique to estimate the low drug concentrations. This will be a major issue following ocular administration, since the vitreal drug concentrations are very low and continuous sampling using a microdialysis system may result in insufficient analyte concentration. In such circumstances, direct coupling of ocular microdialysis to highly sophisticated methods such as LC-MS, MS/MS and NMR techniques, may provide a viable option.

Another exciting approach is simulation model in which ocular distribution after systemic drug administration has been predicted or modeled. According to simulation study, distribution and clearance between vitreous and plasma was obtained from a previous QSPR model for clearance of intravitreal drugs. These values were used in a pharmacokinetic simulation model to depict entry of unbound drug from plasma to vitreous. Reliable predictions were obtained using computational value of ocular distribution clearance, systemic concentrations of unbound drug, and a simple pharmacokinetic model. This approach can be used in drug discovery to estimate ocular drug exposure at an early stage (95).

Overall, the use of pharmacokinetics modeling can significantly reduce the time and cost of formulation development in ocular therapeutics. The development and optimization of the ocular dosage forms can be augmented with pharmacokinetics models during the preclinical phase, and the tentative pharmacokinetics profile can be obtained. This is expected to reduce the number of experiments by providing guidance to the design of dosage forms, in vivo animal experiments and clinical studies. A reliable pharmacokinetics model may allow addressing bioavailability concerns early during ocular drug delivery development and may improve the accuracy significantly.

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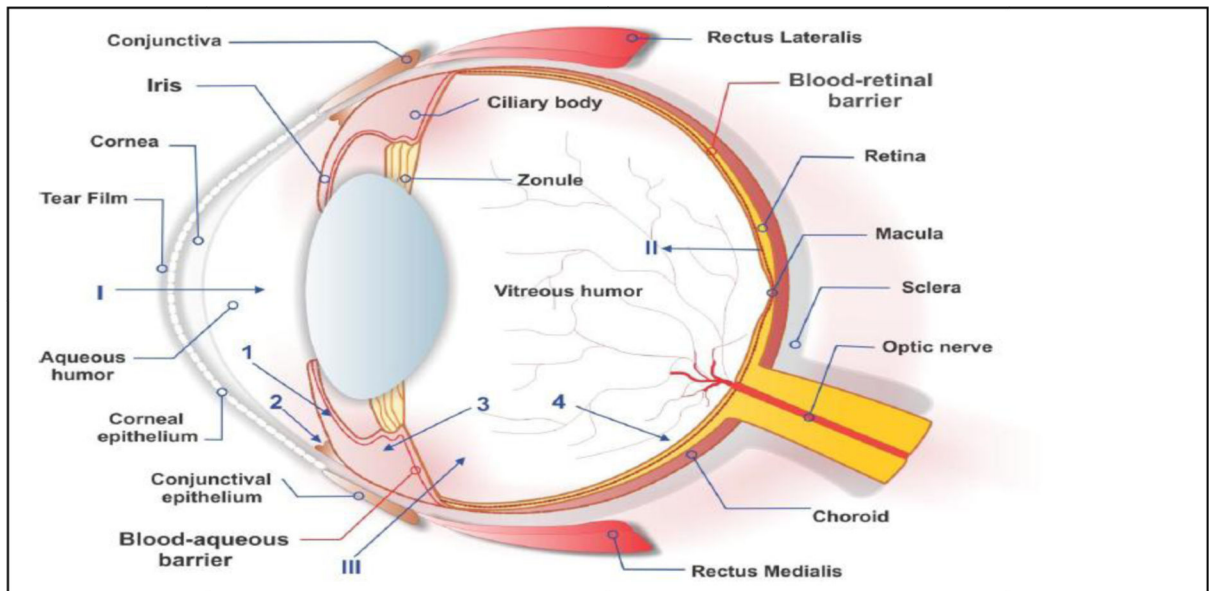
Fig. 1.
Schematic illustration of the main structure of the eye and the ocular barriers. The primary physiologic blockage against installed drugs is the tear film. Cornea is the main route for drug transport to the anterior chamber (I). The retinal pigment epithelium and the retinal capillary endothelium are the main barriers for systemically administered drugs (II). Intravitreal injection is an invasive strategy to reach the vitreous (III). The administered drugs can be carried from the anterior chamber away either by venous blood flow after diffusing across the iris surface (1) or by the aqueous humor outflow (2). Drugs can be removed from the vitreous away through diffusion into the anterior chamber (3), or by the blood–retinal barrier (4). The image was adapted with permission from Reference 10.
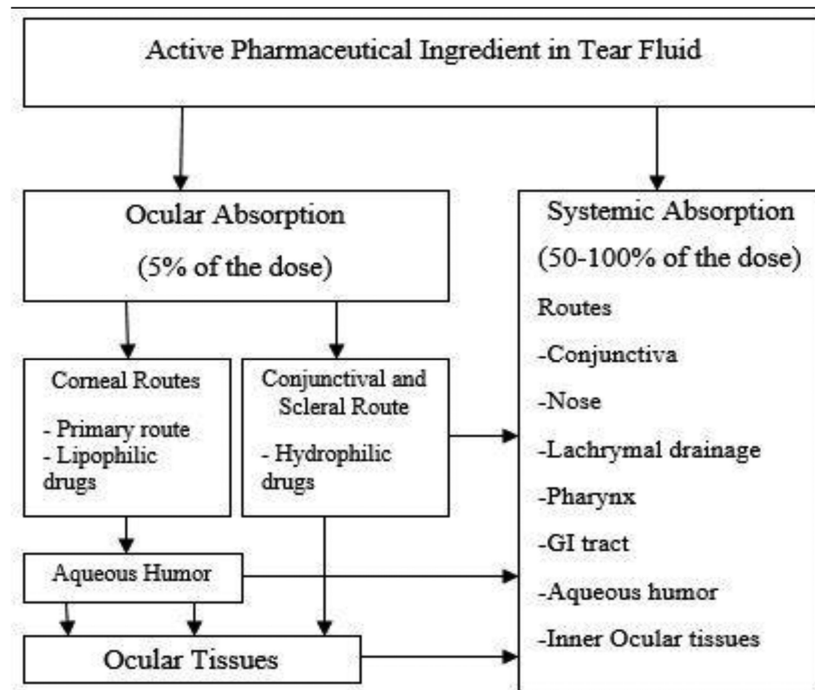
Fig 2.
Schematic representation of ocular absorption through topical administration. The image was adapted with permission from Reference 1.
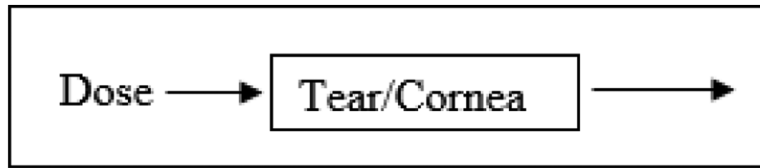
Fig 3.
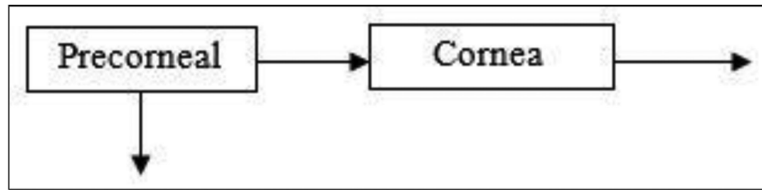Schematic of one compartment pharmacokinetic model

Fig 4.
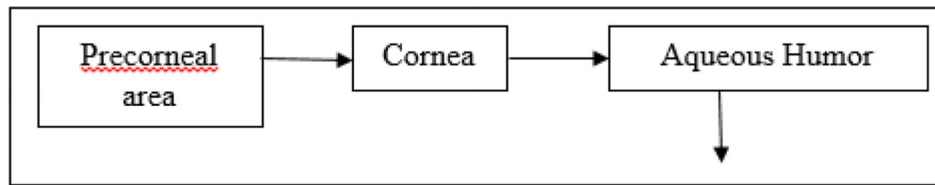Schematic of two compartment pharmacokinetic model

Fig 5.
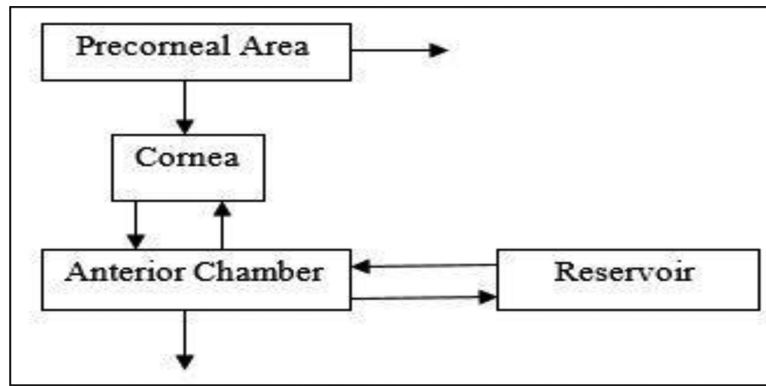Schematic of three compartment pharmacokinetic model

Fig 6.
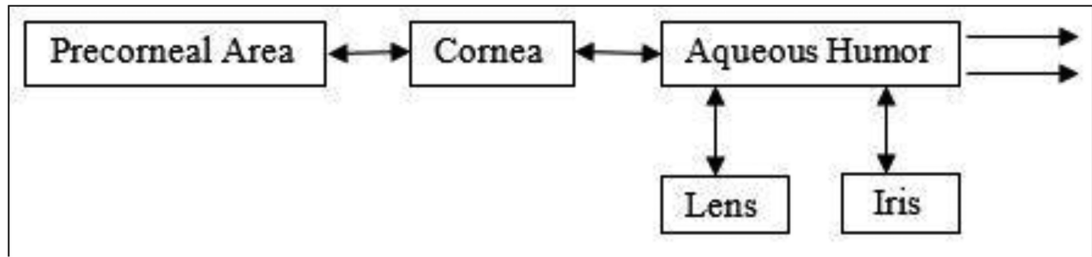Schematic of four compartment pharmacokinetic model

Fig 7.
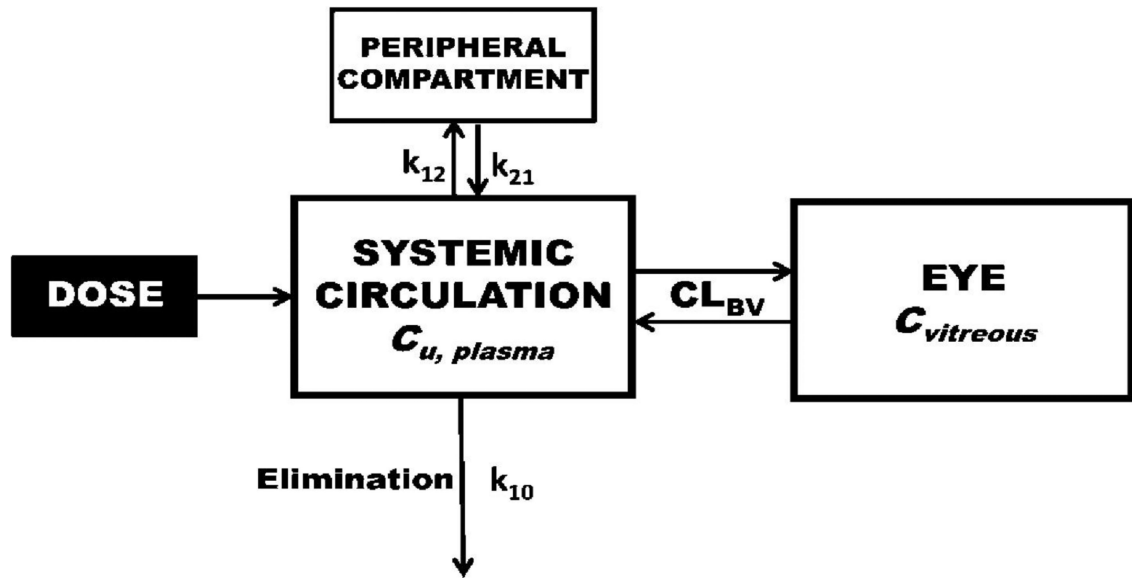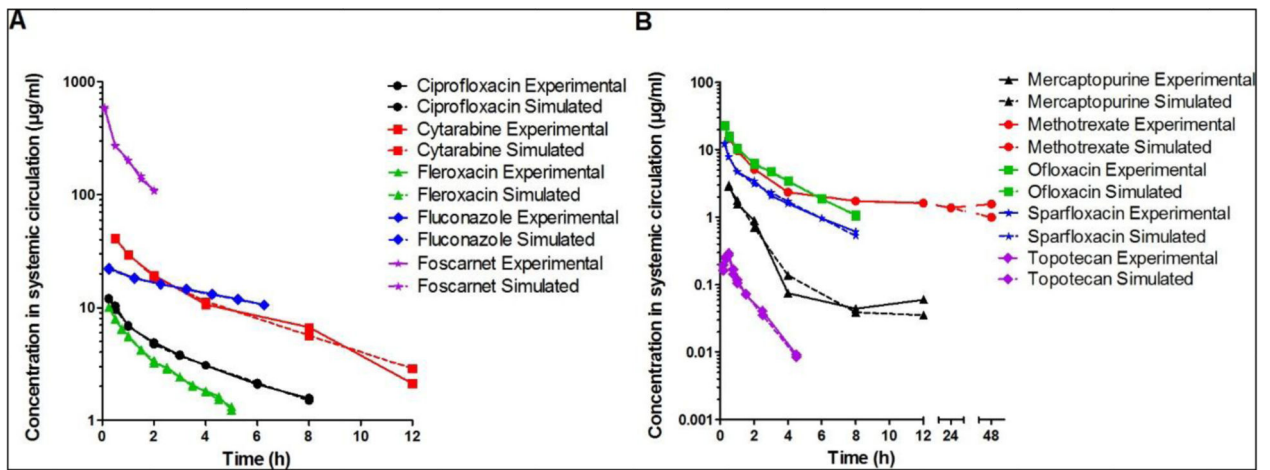Schematic of four compartment pharmacokinetic model

Fig 8.
Compartmental model for drug distribution from systemic circulation to the ocular vitreous.
Reproduced with permission (Ref. 40)

Fig. 9.
Experimental (solid line) and simulated (dashed line) concentrations of the drugs in systemic circulation (plasma or serum) of rabbits after intravenous administration. The drugs ciprofloxacin, cytarabine, fleroxacin, fluconazole, and foscarnet are presented in panel A and mercaptopurine,methotrexate, ofloxacin, sparfoxacin, and topotecan in panel B. Reproduced with permission (Ref. 40)

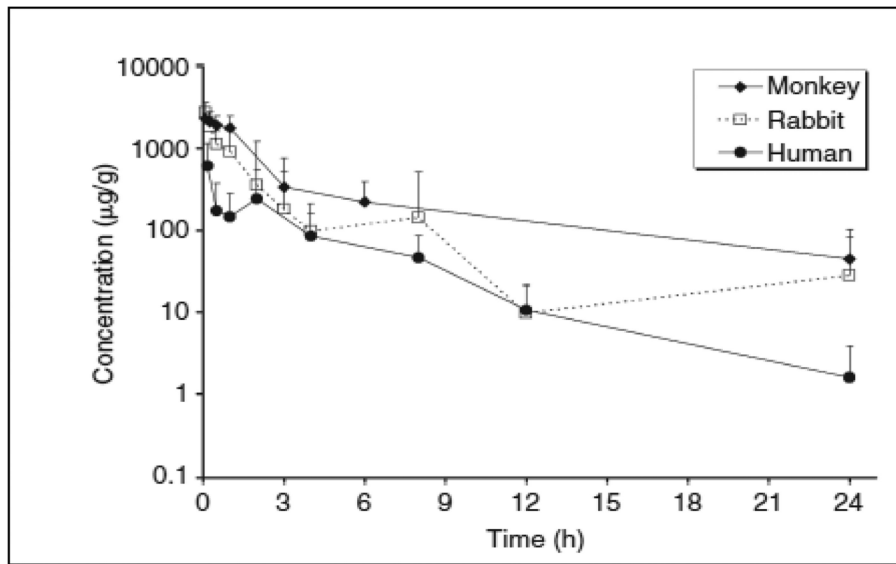**Fig. 10.**
Besifloxacin concentration versus time profiles in tear fluid from cynomolgus monkeys, pigmented rabbits, and humans following single topical ocular administration of besifloxacin ophthalmic suspension (0.6%) (58).
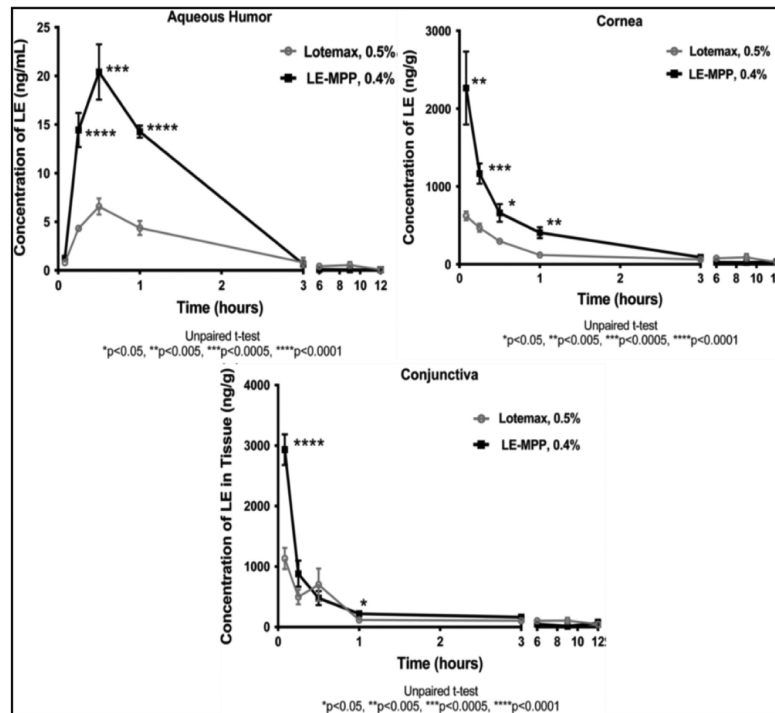
Fig. 11.
Pharmacokinetic profile of loteprednoletabonate in rabbit aqueous humor, cornea and conjunctiva. The value shown for each time point is the mean ± SEM for six samples. Reproduced with permission from Ref. 68
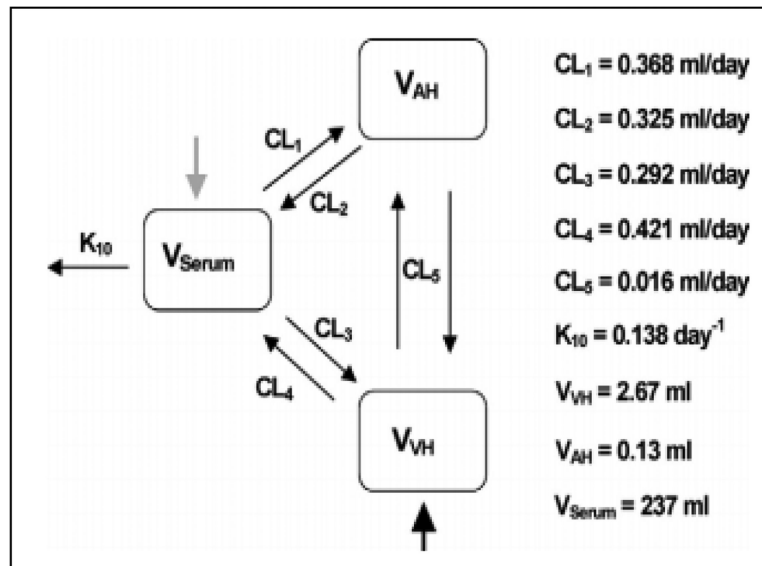
Fig. 12.
Bevacizumab is distributed from the aqueous humor into the serum and vitreous humor. It is also distributed from the serum into the aqueous and vitreous humor, and excreted. CL1 = distribution from the serum intothe aqueous humor; CL2 = distribution from the aqueous humor into theserum; CL3 = distribution from the serum into the vitreous humor; CL4 = distribution from the vitreous humor into the serum; CL5 = distributionbetween the vitreous and aqueous humor; K10 = elimination rate constant;VAH = volume of the aqueous humor; VVH volume of thevitreous humor; V_{Serum}= volume of the serum compartment.
Reproduced with permission from Ref. 71

Table 1

CsA concentrations (ng/g or ngEq/g) in ocular tissues after topical administration in rabbits, dogs and humans (62).

Formulation	Regimen	Conjunctiva	Cornea	Aqueous humor	Retina	Blood
Rabbits						
1% in olive oil	1 drop (7ul) q15min × 6		4000	20	150	BLQ
2% in olive oil	1 drop (100ul) qid for 10 days		1111± 449			89± 60
2% in castor oil	1 drop (10ul)		900-1400	25-45	7.0-18	
0.1% emulsion	1 drop (50ul) bid for 9.5 days	1920	4810	7.1	<1.9	
0.2% emulsion	1 drop (35ul)	1230	751	7		
0.05% emulsion	1 drop (50ul) bid for 9.5 days	713	1550	1.4	<0.7	
0.5% solution	1 drop (35ul) q15min × 4	6920	9410	<59		
Dogs						
0.2% emulsion	1 drop (35ul) bid for 7 days	2007	1890	0.6	1.2	
0.5% solution	1 drop (35ul) q15min × 4	3100	3840	<59		
Humans						
2% in olive oil	2 drops q6h × 4			23.7 ± 9.8		
2% in peanut oil	1 drop tid for 3 days	10 ± 10		33 ± 45		4.7
2% in oil	1 drop			95 ± 140		
0.5% suspension			343-450	<50		<50
0.5% solution	1 drop q15min		3687± 1077	6.1		

Table 2

Pharmacokinetic parameters of azithromycin formulation (65)

Formulation	C_{max} (μg/g)[a]		AUC_{0-144h} (μg*h/g)[a]		T_{max} (h)		$t_{1/2}$ (h)	
	Without polycarbophil	With polycarbophil	Without polycarbophil	With polycarbophil	Without polycarbophil	With polycarbophil	Without polycarbophil	With polycarbophil
Conjunctiva	9.55± 5.12	108± 35.1	79.2± 16.2	737± 93.2	0.083	0.083	48	63
Cornea	8.78± 1.61	40.4± 8.73	196± 20.8	837± 81	0.083	0.083	91	67
Tear Film[b]	893± 215	10539± 1494	155± 28.4	3016± 397	0.083	0.083	37	15
Aqueous humor[b]	0.003± 0.0004	0.076± 0.029	0.250± 0.017	0.689± 0.077	24	0.083	61	80

[a]P 0.05 between formulations for Cmax and AUC in all tissues/fluids.

[b]Tear film and aqueous humor values for Cmax and AUC are reported in μg/mL and μg*h/mL, respectively.

Table 3

Concentrations of bevacizumab in the aqueous, vitreous, and serum after intravitreal injection of 1.25 mg bevacizumab (71).

Compartment	$t_{1/2}$ (days)	T_{max} (days)	C_{max} (µg/ml)	% of Vitreous C_{max}	AUC $_{0-\infty}$ (µg/ml*day)	Exposure to Bevacizumab as a % of Vitreous Exposure
Vitreous	4.32	1	400	-	3300	-
Aqueous	4.88	3	37.7	9.4	295	8.9
Serum	6.86	8	3.33	0.8	54	1.6

Table 4

Pharmacokinetic parameters for brimonidine (anti-glaucoma drug) following single intravitreal injection in normal and diseased dutch belted rabbit model

Ocular tissue	Pharmacokinetic parameter, unit	Normal dutch belted rabbit	Dutch belted rabbit model of glaucoma
Aqueous humor	Cmax, ng/mL	64.46 ±22.4	37.1± 28.7
	AUC0–tlast, ng_h/mL	429± 72	340±51
	Tmax, h	1	2
	T1/2, h	NC	29.7
Retina	Cmax, ng/mL	33,600±14,100	25,500±7,600
	AUC0–tlast, ng_h/mL	134,000±12,000	101,000±13,000
	Tmax, h	0.5	0.5
	T1/2, h	9.90	8.52
Choroid	Cmax, ng/mL	38,500± 11,900	32,200 ±13,000
	AUC0–tlast, ng_h/mL	444,000±38,000	314,000 ±38,000
	Tmax, h	0.5	0.5
	T1/2, h	NC	NC
Aqueous humor	Cmax, ng/mL	1,060±1,370	317
	AUC0–tlast, ng_h/mL	3,720±710	2,030±230
	Tmax, h	1	1
	T1/2, h	4.42	8.51
Retina	Cmax, ng/mL	60,900±14,100	33,000±8,600
	AUC0–tlast, ng_h/mL	359,000±26,000	206,000±21,000
	Tmax, h	2	2
	T1/2, h	2.95	3.77
Choroid	Cmax, ng/mL	53,800±3,100	42,300±27,600
	AUC0–tlast, ng_h/mL	277,000±9,000	185,000±27,000
	Tmax, h	2	2
	T1/2, h	3.30	3.43

Table 5

Enhanced Ocular Permeability of Transporter Targeted Prodrugs

Targeted transporters and tissue /cell line	Drugs/prodrugs	Kinetics	References
B(0,+) on corneal epithelium	L-asp-ACV	4 fold↑ of L-asp-ACV transcorneal permeability	(82)
B(0,+) on corneal epithelium	y-glutamate-ACV	Transporter recognition and higher aqueous solubility of prodrug	(83)
B(0,+) on corneal epithelium	Phe-ACV and y-glutamate-ACV	Prodrug inhibited transport of L-arginine indicating substrate of B(0,+)	(84)
OPT system on corneal epithelium	L-Val-ACV	3fold ↑ of L-Val-ACV	(85)
OPT system on corneal epithelium	Gly-Val-GCV, Val-Val-GCV, and Try-Val-GCV	Significant ↑ transcorneal permeability of these prodrugs by OPT; ↑ AUC and C_{max} of these prodrugs	(86, 87)
OPT system on corneal epithelium	Val-quinidine, Val-Val-quinidine	Prodrugs transported through OPT and circumvented corneal P-gp mediated efflux	(88)
OPT system on retinal epithelium	Gly-Val-GCV, Val-Val-GCV, and Try-Val-GCV	2 fold ↑ of prodrugs across retinal epithelium and mediated by OPT	(89)
SMVT on retinal epithelium	Biotin-GCV	Permeability of biotin-GCV ↑ into retina-choroid and slow elimination from vitreous	(90)
SMVT on corneal epithelium	Biotin-ACV	4.6 fold ↑ accumulation of biotin-ACV in human corneal epithelium through SMVT.	(91)

OPT: oligopeptide transporter; SMVT: sodium dependent multivitamin transporter; B(0,+): amino acid transporter; ACV: acyclovir; GCV: ganciclovir. This Table is adapted and modified from Gaudana et al, 2010. (8)