Chapter 3
Drug Transport Across Blood-Ocular Barriers and Pharmacokinetics

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Abstract Systemically administered drugs do not reach or have limited diffusion into the eye due to the presence of various ocular barriers. Therefore, intravitreal, intracameral, subconjunctival and sub-tenon routes are the preferred options for directly injecting drugs to into the eyes or ocular structures. Presence of drug transporters in the ocular or retinal barriers play a vital role in the ocular pharmacokinetics of the drugs administered by systemic or direct injection routes. This chapter discusses the involvement of various transporters in providing barrier functions for the transport of drugs in and out of eye. It also discusses about the general principles regarding ocular pharmacokinetics of drugs applied systemically and topically. Studies revealing the functional importance of transporters in barriers and models developed to predict the ocular kinetics of drugs, pharmaceutical factors, ocular drug metabolism and elimination are discussed to give further understanding while selecting a suitable drug for ocular therapy.

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3.1 Blood-Ocular Barriers

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3.1.1 Introduction

The objective of medical therapy is to achieve the best favorable effects of drugs and to avoid their undesirable, unwanted side effects. The principles of pharmacokinetics – absorption, distribution, metabolism or biotransformation, and elimination – constitute a key knowledge for the appropriate choice and clinical success of therapeutic drug regimens (Buxton and Benet 2011). Unlike the vast majority of human organs, the eye is relatively excluded from the access of systemic circulating blood by several barriers (Jordan and Jordán and Ruíz-Moreno 2013). Therefore, direct administration of drugs in the eye, either topically or by ocular injection, is considered as specific routes of drug administration (Jordán and Ruíz-Moreno 2013). In order to have pharmacological activity, a drug must be present at the local site of action, which, in turn, requires its absorption and distribution. Absorption refers to the extent of drug reaching the systemic blood circulation or the central compartment (Buxton and Benet 2011). For oral administered drugs, several variables influence the absorption, such as the physical state of the drug, the blood flow at the local absorption, and the presence of ionized/nonionized forms (Buxton and Benet 2011). However, bioavailability is the concept reflecting the amount of drug reaching the local action after the absorption process and constitutes the most clinically meaningful pharmacokinetic concept (Buxton and Benet 2011).

During their passage through the body, drugs have to cross several cell membranes. This process may occur by passive transport (paracellular transport or diffusion) or by active transport (facilitated diffusion or drug transporters) (Buxton and Benet 2011). The later involves the active participation of molecular cell structures (Buxton and Benet 2011). It usually occurs as a movement against a concentration gradient and is an energy-consuming process (Buxton and Benet 2011).

The treatment of several eye diseases is based on the topical administration of drugs. They may act at the ocular surface or may need to cross epithelial cells (cornea, conjunctiva, or both) to reach local action (Attar et al. 2005). Transporters, particularly of peptide nature, are present in those tissues and regulate the action of several drugs (Attar et al. 2005). Drug-metabolizing enzymes have also been characterized in ocular tissues, with drug metabolism induction and inhibition properties as well as polymorphisms, particularly in cytochrome P450 expressions (Attar et al. 2005).

However, systemic administered drugs do not reach or have limited diffusion in the eye, due to the presence of ocular barriers, giving place to the need of directly inject drugs (intravitreous administration) (Jordán and Ruíz-Moreno 2013).

3.1.2 Ocular Barriers to the Penetration of Systemically Administered Drugs

The situation in the blood-ocular barriers is better understood if we consider two main barrier systems in the eye. One, regulating exchanges between blood and the intraocular fluids and involving a variety of structures, concerns the primary and ciliary body and is called the blood-aqueous barrier. Here, inward movements from the blood into the eye predominate. The aqueous humor is secreted into the posterior chamber by the ciliary processes from where it flows through the pupil into the anterior chamber and leaves the eye by bulk flow at the chamber angle by the trabecular or uveoscleral routes. There are diffusional solute exchanges between the aqueous humor and the surrounding tissues, the posterior chamber, and the vitreous compartment (Adler 1962; Bito 1977; Cunha-Vaz and Maurice 1967).

The other barrier, particularly tight, where outward movement from the eye into the blood appears to predominate and where the penetration into the eye of only a few important metabolic products in allowed, is called the blood-retinal barrier. It is responsible for homeostasis of the neuroretina.

3.1.3 Ocular Barriers to the Penetration of Topically Instilled Drugs

3.1.3.1 The Blood-Aqueous Barrier (BAB)

The blood-aqueous barrier is formed by the nonpigmented epithelium of the ciliary body, the posterior iris epithelium, the endothelium of the iris vessels with junctions of the leaky type, and the endothelium of Schlemm's canal (Chen et al. 2008). The BAB excludes aqueous and vitreous humors from blood plasma proteins to avoid compromising the transparency of intraocular fluids and to maintain their osmotic and chemical equilibrium (Butler et al. 1988). The aqueous, which is the fluid in the anterior and posterior chambers, is produced by the nonpigmented ciliary epithelium of the ciliary body (Chen et al. 2008). The aqueous humor maintains a normal homeostatic environment and is essential to the proper functioning of anterior chamber tissues (Chowdhury et al. 2010). The reason for a different composition of the aqueous humor comparing to the plasma resides in two physiological characteristics of the anterior segment: the blood-aqueous barrier and the active transport of various organic and inorganic substances by the ciliary epithelium. The greatest differences are the low protein and high ascorbate concentrations in the aqueous relative to plasma (200 times less and 20 times greater, respectively) (Gabelt and Kaufman 2011).

The BAB supports the nutrition and function of the cornea and lens (Chen et al. 2008). Ocular inflammation, intraocular surgery, trauma, or vascular diseases may cause alterations in the BAB (Chen et al. 2008). The aqueous becomes

cloudy due to leakage of plasma proteins into the posterior and anterior chambers. The presence of fibrinogen and other proteins may turn the aqueous plasmoid (Hosoya and Tomi 2005). When the breakdown of the BAB occurs, inflammatory cells may be present in the aqueous. However, for BAB the expression of drug transporters and metabolizing enzymes has not been characterized; therefore, its role on ocular drug kinetics lacks elucidation (Chen et al. 2008; Urtti 2006).

The BAB is not as able as the BRB in respect to limiting molecular diffusion (Occhiutto et al. 2012). It was identified that after intravenous injection, substances such as inulin, chloride, sucrose, phosphate, potassium, sodium, urea, proteins, and some antibiotics could be found on the anterior side of the vitreous humor resulting from the ciliary circulation. However, these substances could not reach the retina directly due to the blood-retinal barrier (Occhiutto et al. 2012).

3.1.3.2 The Blood-Retinal Barrier (BRB)

The BRB is a physiological barrier that regulates ion, protein, and water flux into and out of the retina and prevents leakage into the retina of macromolecules and other harmful agents (Cunha-Vaz 1979, 2004). This barrier, which has many similarities to the blood-brain barrier, is essential to the integrity of the retina, and once BRB damage occurs, vision may become impaired (Cunha-Vaz 1997, 1979, 2010).

The BRB consists of inner and outer components (inner BRB [iBRB] and outer BRB [oBRB]) (Cunha-Vaz 2010). It provides metabolic support to the neural and glial cells through a unique vascular structure while minimizing interference with light sensing (Runkle and Antonetti 2011). The oBRB is constituted by the retinal pigment epithelium and is located at the posterior of the eye, controlling exchange of nutrients with the choroidal vessels (Runkle and Antonetti 2011). The vascular and epithelial components of the blood-retinal barrier maintain the specialized environment of the neural retina (Runkle and Antonetti 2011).

3.1.3.3 The Inner Blood-Retinal Barrier (iBRB)

The iBRB is established by the tight junctions (zonulae occludentes) between retinal endothelial cells (Cunha-Vaz 2010). The retinal endothelial layer functions as an epithelium and is directly associated with its differentiation and with the polarization of BRB function (Cunha-Vaz 2010). This continuous endothelial cell layer, which forms the main structure of the iBRB, rests on a basal lamina that is covered by the processes of astrocytes and Müller glia cells (Cunha-Vaz 2010). Pericytes are also present in the iBRB, encased in the basal lamina, in contact with the endothelial cells, but they do not form a continuous layer and therefore do not contribute to the diffusional barriers (Jordán and Ruíz-Moreno 2013). Astrocytes, Müller cells, and pericytes are considered to affect maturation and maintenance of the

BRB by transmitting regulatory signals to endothelial cells indicating changes in the microenvironment of the retinal neuronal circuitry (Cunha-Vaz 2010; Schlosshauer 2007).

The vascular endothelium found in the adult retina and the brain shows similar structural characteristics, though some differences have been identified, such as a higher density of interendothelial junctions and the lack of g-glutamyl transpeptidase (gGT) in the retinal endothelium (Schlosshauer 2007; Vinores 1995). The development of the endothelial network and the formation of the iBRB are characterized by a primary construction phase followed by a secondary destruction period until the adult layout is sculptured (Schlosshauer 2007). Furthermore, the initial vascular network is leaky. The expression of barrier characteristics appears to be one of the latest steps during maturation (Schlosshauer 2007).

Retinal Vascular Endothelial Cells

The retinal vascular endothelial capillaries are composed by non-fenestrated cells which have a paucity of vesicles (Cunha-Vaz 2010). Such vesicles promote receptor-mediated processes of endocytosis or transcytosis. Other mechanism for diffusion of substances across the BRB is the channel-facilitated transport using transmembrane proteins, such as the glucose transporter GLUT1 which supplies glucose to the neuronal tissue (Cunha-Vaz 2010). The disruption of the iBRB in pathological conditions is associated with increased vesicle formation and disrupted endothelial membranes and may develop before opening of the tight junctions being detected (Cunha-Vaz 2010).

Retinal Vascular Endothelial Tight Junctions

The main function of the tight junctions, TJs, is the ion, water, and nutrient flow regulation between the retina and blood vessels, as well as the protection of the neural retina from inflammatory cells and their toxic products found in the systemic circulation (Vinores 1995; Gardner et al. 2002; Kaur et al. 2008). Tight junctions may also serve as regulatory centers that can help to coordinate several cell processes, such as the regulation of cell morphology, proliferation, and establishment and maintenance of apico-basal polarity (Kaur et al. 2008).

The TJs of the retinal vascular endothelium are formed by fusion of the outer leaflets of adjacent endothelial cell membranes (Fernandes et al. 2012). The TJ complex contains at least 40 transmembrane proteins and internal adapter proteins that regulate paracellular flux (Fernandes et al. 2012). Transmembrane proteins constituting the TJ are occludins, claudins, and junctional adhesion molecules. Adapter proteins are localized below the membrane and act as TJ organizers and cytoskeleton anchors (Fernandes et al. 2012). The TJs are regulated by signal transduction through cyclic AMP levels, or tyrosine kinases, for example (Fernandes et al. 2012).

Müller Glia Cells, Astrocytes, and Pericytes

The close spatial relationship between Müller glia cells and blood vessels in the retinal suggests that these cells have a critical role in the formation and maintenance of the BRB by regulating the functions of the barrier cells in the uptake of nutrients and in the disposal of metabolites under normal conditions (Cunha-Vaz 2010). Muller cells have matrix metaloproteinases that promote proteolytic degradation of TJ proteins occludins.

Astrocytes in the iBRB play a critical role during normal inner retinal vascularization (Dorrell et al. 2002; Fruttiger et al. 1996; Provis et al. 2000), and degeneration of retinal astrocytes in ischemic tissues is associated with failure of the blood-retinal barrier in oxygen-induced retinopathies (Chan-Ling and Stone 1992; Dorrell et al. 2010). These cells are originated from the optic nerve and migrate to the retinal nerve fiber layer during retinal vascular development, to closely associate with the retinal vessels helping to maintain their integrity (Cunha-Vaz 2010). Astrocytes enhance the expression of the TJ protein Z0.1 which has a role in moderate TJ integrity (Cunha-Vaz 2010).

Pericytes support endothelial cells by secreting angiopoietin 1, which induces the protein expression of occlud and other proteins integrating TJ. These cells help in the regulation of vascular tone, secretion of extracellular materials and in the process of phagocytosis (Cunha-Vaz 2010).

3.1.3.4 The Outer Blood-Retinal Barrier (oBRB)

The outer blood-retinal barrier is formed by a single layer of retinal pigment epithelial (RPE) cells that are interconnected in their apical side by TJs (zona occludens) (Fernandes et al. 2012). The permeable Bruch's membrane separates the RPE from the overlying fenestrated choriocapillaris (Fernandes et al. 2012). The RPE is fundamental to regulate the access of nutrients from the blood to the photoreceptors and in the elimination of waste products and maintenance of retinal adhesion (Cunha-Vaz 2010). Besides these features that serve the (outer) retina in general, the metabolic relationship between the RPE villi and the photoreceptors is considered critical for the maintenance of visual function.

RPE Cells

RPE cells regulate water content and lactic acid removal generated by the characteristic high metabolic rates in the retina (Jordán and Ruíz-Moreno 2013). The adhesion of the retina to the RPE is based on the interphotoreceptor matrix, which is synthesized by the RPE (Steinberg and Wood 1979). The viscosity and bonding properties of the matrix are dependent on its hydration and ionic composition, both of which are controlled by vectorial flux of water and selected ions. The net effect of several RPE pump systems is a movement of water across the RPE in the

apical-to-basal, i.e., retina-choroid, direction (Zauberman 1979). The water transport is linked with ion transport, organic anion transport, and other drainage mechanisms (Cunha-Vaz 2010).

The RPE is further involved in photoreceptor outer segment renewal (Fernandes 2012). RPE cells also transport glucose and retinol from blood to the photoreceptors (Cunha-Vaz 2010).

RPE Tight Junctions

The TJs of the RPE cells are anchored to the actin cytoskeleton of RPE cells, interact with signaling molecules, and are important for the establishment of cell polarity (Cunha-Vaz 2010). These junctions restrict the paracellular movement of larger molecules between neighboring RPE cells (Cunha-Vaz 2010). As the retinal vascular endothelium TJs, occludins, claudins, and adapter proteins have been detected in the RPE TJs (Cunha-Vaz 2010). The TJ complexes of the iBRB and oBRB allow the establishment of the polarities of the BRB, restricting paracellular diffusion of blood-barrier compounds in the neuronal tissues (Cunha-Vaz 2010).

3.1.3.5 BRB and Ocular Immune Privilege

The immune response has developed and evolved to protect the organism from invasion and damage by a wide range of pathogens. With time, the immune system has developed destructive responses that are specific for pathogens as well as tissues. However, such tissue injury may have a devastating effect on the function of an organ such as the eye, which needs to maintain optical stability (Cunha-Vaz 2009).

The existence of ocular immune privilege is dependent on multiple factors such as immunomodulatory factors and ligands, regulation of the complement system within the eye, tolerance-promoting antigen-presenting cells (APCs), unconventional drainage pathways, and, with particular relevance, the existence of the blood-ocular barriers (Cunha-Vaz 2009).

The blood-ocular barriers provide a relative sequestration of the anterior chamber, vitreous humor, and neurosensory retina from the immune system and create the necessary environment for the existence of ocular immune privilege (Fernandes 2012). The evolution of immune privilege as a protective mechanism for preserving the function of vital and delicate organs such as the eye has resulted in a complex system with multiple regulatory safeguards for the control of both innate and adaptive immunity (Cunha-Vaz 2009). The consequences of inadvertent bystander tissue destruction by antigen-nonspecific inflammation can be so catastrophic to the organ or host that a finely tuned regulatory system is needed to ensure the integrity of the ocular tissues and maintain optical relationships (Fernandes 2012; Cunha-Vaz 2009).

There are also several lines of evidence that point to immunosuppressive functions in BRB cells, RPE cells, and retinal endothelial cells. These immunosuppressive effects are apparently due to the secretion of a variety of soluble factors, such as cytokines and growth factors (Cunha-Vaz 2009).

3.1.3.6 Regulation of the Microenvironment of the Retina: BRB Transport Systems

The presence of TJ in the BRB prevents free diffusion of polar nutrients essential for the metabolism of the retina, and therefore, the BRB must contain specific transport proteins that are expressed at the plasma membranes of the retinal epithelial and endothelial cells. Epithelial (oBRB) and endothelial (iBRB) cells exhibit a polarized expression of transport carriers in the apical/luminal and basolateral/abluminal plasma membranes (Fernandes 2012). The orientation of these carriers results in preferential blood-to-retina influx or retina-to-blood efflux transport of substrates or in facilitated transport in either direction depending on the concentration gradient of the solutes across the BRB (Fernandes 2012).

Potential routes for facilitated transport across the BRB are the blood-to-retina influx transport system that acts as an energy supply system for the retina, since the iBRB supplies metabolic substrates from the circulating blood, such as glucose, amino acids, vitamins, and nucleosides, to the retina (Fernandes 2012). The other is the retina-to-blood efflux transport system that acts to get rid of hydrophobic xenobiotics and neurotransmitter metabolites (Fernandes 2012).

3.1.3.7 Energy Transport System

The blood-to-retina influx transporters operating at the iBRB supply hydrophilic substrates to the retina. The retina, one of the most metabolically active tissues in the body, uses glucose as its main energy source. Being a hydrophilic molecule, glucose does not freely cross the barrier and its transport from blood into retina is mediated by facilitative glucose transporters, named GLUT1 (Takata et al. 1992; Fernandes et al. 2003). The distribution of GLUT1 at the iBRB is asymmetric, being its expression at the abluminal membrane approximately two- and threefold greater than that at the luminal membrane in humans and rats, respectively (Takata et al. 1992; Fernandes et al. 2003). The higher density of GLUT1 on the abluminal membrane of the retinal endothelial cells suggests that glucose transport is limited at the blood-luminal rather than the abluminal-interstitial interface. In experimental animal models of type 1 and type 2 diabetes, as well as in vitro studies where endothelial cells were exposed to elevated glucose, it has been shown that there is a downregulation of GLUT1 in retinal endothelial cells (Badr et al. 2000; Fernandes et al. 2004).

Besides glucose, dehydroascorbic acid (DHA), which is an oxidized form of vitamin C, is rapidly transported across the BRB via GLUT1 and accumulates as ascorbic acid in the retina. DHA uptake by GLUT1 is competitively inhibited by D-glucose (Minamizono et al. 2006). DHA transport from the blood to the retina

decreases with increasing blood D-glucose concentration under diabetic conditions due to the inhibition of DHA uptake by GLUT1 at the BRB (Minamizono et al. 2006). This can lead to the increased oxidative stress observed in diabetic retinas.

In addition to D-glucose, L-lactic acid appears to be required as an energy source in photoreceptors (Poitry-Yamate et al. 1995), and the transport of this solute between retina and blood seems to be mediated by the monocarboxylate transporter 1 (MCT1). Immunoreactivity for this transporter was found both in luminal and abluminal membranes of rat endothelial cells (Gerhart et al. 1999). It has been shown that the uptake of labeled L-lactic acid is inhibited by protonophores, MCT inhibitors, and a number of other monocarboxylates and monocarboxylic drugs, such as salicylic and valproic acids (Alm and Törnquist 1985; Hosoya et al. 2001). These results suggest that these transports are an attractive route for monocarboxylate drug delivery to the posterior segment of the eye.

Creatine, which plays an essential role in supporting ATP homeostasis in the retina, is transported by creatine transporter (CRT) (Nakashima et al. 2004). The storage and administration of phosphate-bound energy is mediated by the conversion of creatine to phosphocreatine. It has been found to be an asymmetrical distribution of CRT at luminal and abluminal membranes of rat retinal endothelial cells (Nakashima et al. 2004).

In glycolysis, glucose is oxidized to either lactate or pyruvate. Both glycolysis and the tricarboxylic acid cycle generate energy in the form of ATP. Therefore, GLUT1, MCT1, and CRT transporters may act in synergy to maintain energy homeostasis in the retina.

3.1.3.8 Nucleoside Transport System

Adenosine is an important intracellular signaling molecule that is involved in retinal neurotransmission, blood flow, vascular development, and response to ischemia trough cell surface adenosine receptors (Ghiardi et al. 1999; Lutty and McLeod 2003).

The expression at the mRNA level of several equilibrative nucleoside transporters ENT1, ENT2, CNT1, and CNT2 has been detected in retinal endothelial cells (Nagase et al. 2006). By regulating the concentration of adenosine available to cell surface receptors, these transporters influence the retinal physiological processes mentioned above. ENT2 is also responsible for the uptake of some antiviral and anticancer nucleoside drugs (Yao et al. 2001; Baldwin et al. 2004). This has led to the hypothesis that ENT2 at the iBRB could be a potential route for delivering nucleoside drugs from the circulating blood to the retina.

3.1.3.9 Organic Anion Transport System

In order to maintain a constant milieu in the neural retinal, the BRB also carries out the efflux transport of harmful substances like neurotransmitter metabolites, toxins, and xenobiotics (Cunha-Vaz and Maurice 1967).

Members of the family of organic anion-transporting polypeptides (OATP) mediate the Na + -independent transport of a wide range of amphipathic organic compounds, including bile salts, organic dyes, steroid conjugates, thyroid hormones, anionic oligopeptides, numerous drugs, and other xenobiotic substances (Hagenbuch and Meier 2003). Transporters of the family of OATP (OATP2 and OATP14) have been identified in the rat inner and outer BRB (Gao et al. 2002).

3.1.3.10 ABC Transporters

ABC transporters (ATP-binding cassette) are a superfamily of membrane proteins that play a major role in restricting the bioavailability of many drugs in various tissues by pumping agents (with consumption of ATP) from the lipid bilayer or cytoplasm back into the extracellular fluid (Mannermaa et al. 2006). In the ocular tissues, the ABC transporters of greatest significance for efflux transport are P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRP), and breast cancer protein (BCRP) (Mannermaa et al. 2006).

The ABC superfamily is subdivided into seven subfamilies based on similarities in domain structure, nucleotide-binding folds, and transmembrane domains (Dean et al. 2001). The general structure of ABC transporters is composed of 12 transmembrane regions, split into two halves, each with a nucleotide-binding domain (NBD) (Altenberg 2004).

P-gp (MDR1) mediates the efflux of a wide range of drugs from the intracellular to the extracellular space (Fojo et al. 1987). The list of its substrates/inhibitors is continually growing and includes anticancer agents, antibiotics, antivirals, calcium channel blockers, and immunosuppressive agents (Altenberg 2004). P-gp has been shown to be expressed in rat retinal capillaries and cultures of rat retinal endothelial cells (Greenwood 1992; Shen et al. 2003; Hosoya and Tomi 2005). The P-gp-mediated drug efflux pump on the apical plasma membrane of the conjunctiva plays a role in restricting the conjunctival absorption of some lipophilic drugs and xenobiotics.

BCRP (ABCG2) has only one ABC and six putative transmembrane domains, being referred to as a half-ABC transporter, most likely functioning as a homodimer (Krishnamurthy and Schuetz 2006) ABCG2 shows great affinity not only for drugs but also for phototoxic compounds that can cause light-induced damage to the retina (Boulton et al. 2001). BCRP was found to be present in the luminal membrane of mouse capillary endothelial cells by immunolabeling and was shown to be expressed in mouse and rat retinas (Asashima et al. 2006).

These drug efflux pumps at the BRB could act by restricting the distribution of xenobiotics, including drugs and phototoxins, in the retina. Modulation of such efflux mechanisms in conjunction with the treatment of ocular tissues in retinal diseases remains a major challenge.

3.1.3.11 Relevance of the Blood-Retinal Barrier in the Treatment of Retinal Diseases

When administered systemically, drugs must pass the BRB in order to reach therapeutic levels in the retina. Drug entrance into the retina depends on a number of factors, including the plasma concentration profile of the drug, the volume of its distribution, plasma protein binding, and the relative permeability of the BRB (Cunha-Vaz 1979). To obtain therapeutic concentrations within the retina, new strategies must be considered such as delivery of nanoparticles, chemical modification of drugs to enhance BRB transport, coupling of drugs to vectors, etc. (Cunha-Vaz 2010; Fernandes et al. 2012). The BRB must be considered as a dynamic interface that has the physiological function of specific and selective membrane transport from blood to retina and active efflux from retina to blood for many compounds, as well as degradative enzymatic activities (Cunha-Vaz 2009). Better understanding of the transports systems at the BRB will be extremely useful for drug design. Efflux pumps must be effectively circumvented to enhance drug absorption across the retina (Cunha-Vaz 2010). Modulating a drug substrate targeting an influx transporter offers great potential. In this strategy, drugs must be designed such that the modified compounds become substrates of nutrient transporters, leading to enhanced absorption across the ocular barriers. In addition, efflux is effectively circumvented due to diminished or no affinity of the drug molecule toward efflux pumps due to structural modification and binding to the influx transporter (Cunha-Vaz 2010).

Eyedrops are now being developed for the treatment of posterior segment diseases. However, they are generally considered to be of limited benefit. Newer prodrug formulations that achieve high concentrations of the drug in the posterior segment may have a role in the future. Meanwhile, periocular injection is one modality that has offered mixed results (Cunha-Vaz 2010; Fernandes et al. 2012).

Finally, recent years have seen a generalized and surprisingly safe utilization of intravitreal injections, a form of administration that circumvents the BRB. Steroids and a variety of anti-vascular endothelial growth factor (anti-VEGF) drugs have been administered through intravitreal injections to a large number of patients without significant side effects and demonstrating good acceptance by the patients. Intravitreal injections can achieve high drug concentrations in the vitreous humor and retina, preserving BRB integrity and its crucial protective function (Cunha-Vaz 2010). At present, the major challenge appears to be the need to decrease the number of intravitreal injections, which, in the case of anti-VEGF treatments, are given every 6 weeks to maintain efficacy. The search for safe slow-delivery devices or implantable biomaterials is ongoing, but the invasive approach to retinal disease treatment appears to be an effective way of rapidly reaching therapeutic levels in the retina in the presence of a functioning BRB (Cunha-Vaz 2010; Fernandes et al. 2012).

3.1.4 Conclusion

It has been shown to be particularly difficult to reach therapeutic drug concentrations in the retina using traditional routes of administration, other than the exception of intravitreal injections. Topical treatments generally present favorable benefit/risk ratios but have been limited to ocular anterior segment pathologies. The BRB is an obstacle to drug penetration and circulation within the retina, since transporters expressed by this barrier play a decisive role on drug bioavailability to this tissue.

3.2 Ocular Pharmacokinetics and Factors Affecting Ocular Disposition of Drugs

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Drug transfer across the ocular barriers is expected to govern the pharmacokinetics of drugs which in turn affect their dynamic properties. Drug entry into the ocular structures is conventionally enabled by systemic, intraocular (intracameral and intravitreal), periocular (sub-tenon and subconjunctival), and topical administrations. Except direct injection into the ocular compartments such as intravitreal or intracameral, all other routes for ocular drug administration would be governed by their systemic pharmacokinetics where they behave as sites for drug absorption.

Systemic drugs seldom reach effective concentration (above ED50 or MIC 90) into normal ocular tissues. Therefore, systemic drug routes are not preferred for most of the ocular diseases. Failure to reach adequate drug level into ocular structures can be attributed to their therapeutic failure at many instances. Typically, eye has been reported to have two predominant pathways of drug elimination. After direct injection some drugs are eliminated from vitreous through retinal pathway (Posterior) and some of them are reported to be eliminated through anterior pathway. Hence, ocular disposition of drugs can play an independent role unlike other places where they are not guarded by blood organ barriers.

3.2.1 Pharmacokinetics of the Topically Applied Drugs

Pre-corneal factors are the key determinants of the absorption of the topically administered drugs into the eye. Based on the port of entry, pharmacokinetics of the drugs into anterior and posterior segments of the eye can be classified. If absorption takes place through the cornea or conjunctiva after topical instillation of drugs such as eyedrops, the levels reaching aqueous humor are suggestive of the extent of its

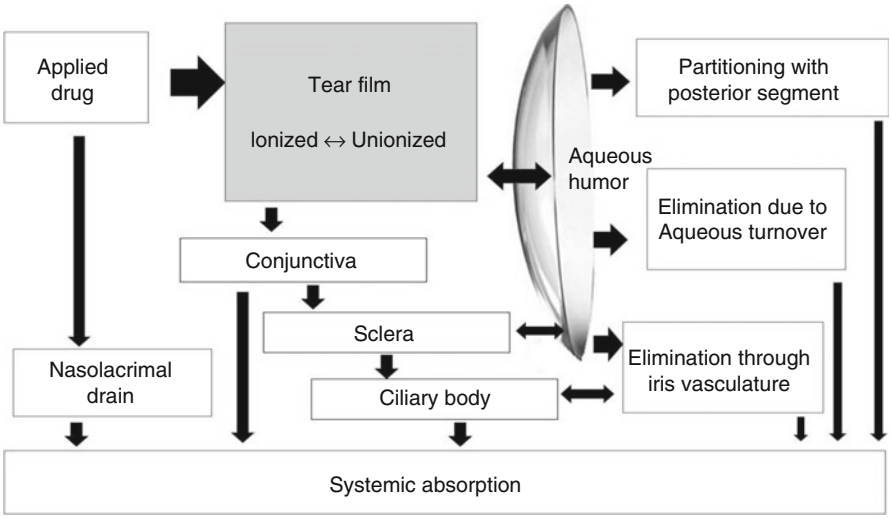

Fig. 3.1 Schematic representation of the fate of topically applied drugs on the eye and their disposition

penetration. The elimination kinetics of the drug from the aqueous humor is favored through anterior pathways of elimination including aqueous drainage, absorption into iris vasculature, re-equilibration with corneal tissues, and reverse draining to the posterior segment through retinal blood vessels (Fig. 3.1). In most of the cases, the former route is favored than the later. Effect of coadministered drugs are also reported to influence ocular absorption of drugs. Topically instilled anesthetic agents significantly suppressed the rate of tear turnover and thereby increased pre-corneal drug residence time (Patton and Robinson 1975).

3.2.2 Pre-corneal Factors Affecting Ocular Penetration of Drugs after Topical Application

In the air-corneal interface, one would see several layers of physiological importance involved in the maintenance of corneal integrity and transparency very efficiently. The outer most layer is a mono lipid layer floating on the tear film having hydrophobic lipids toward air and amphiphilic lipids in the lipid-water interface thereby giving integrity. These lipids are secreted by meibomian glands, which are embedded in the tarsal plate of the upper and lower eyelids giving protection against aqueous evaporation. The cornea produces a small proportion of the aqueous layer as well as mucins in the glycocalyx. The conjunctiva secretes substantial electrolytes and water into the aqueous layer and mucins into the mucous layer. At the same time, the conjunctiva can also absorb electrolytes, water, and applied drugs from the tear film thereby modifying their pharmacokinetics. Although five layers

of the cornea are very distinct, the corneal epithelium, stroma, and endothelium are the layers that gain pharmacological importance as far as drug penetration is concerned. In these layers, drug transport across the cornea can be modulated by drug transporters present in the epithelium and endothelium. However, the resistance for the transport of the drug across these membranes is also affected by the physiochemical nature of the molecule. The corneal epithelium is hydrophobic and the stroma is hydrophilic; thereby both water-soluble and lipid-soluble drugs cannot penetrate freely. The cornea behaves as a typical biological membrane in which most of the drugs cross this structure either by intracellular or transcellular diffusion (Lee and Robinson 1986).

Topically applied eyedrops exceeding the volume of 20 μl would be drained from the cul-de-sac via nasolacrimal duct or removed externally by blinking. Application of low volumes of eyedrop would be beneficial for getting better bioavailability. Volume of solution delivered by commercial eye droppers can be between 25–50 μl and if the subject is not blinking, eye can hold upto 30 μl if instilled carefully. However, reflex blinking may increase both solution drainage and overflow from the conjunctival sac. Decreasing the volume of instillation is expected to improve risk-to-benefit ratio of the potent compounds known to have systemic toxicity (Lynch et al. 1987).

Most of the topically applied drugs are the salts of acids or bases like ciprofloxacin HCl, timolol maleate, prednisolone acetate, pilocarpine trinitrate, atropine sulfate, bromfenac sodium, etc. Drug molecules in the tear film can be influenced or get influenced by the tear film pH depending on their concentration and property. Therefore, the extent of their ocular bioavailability would depend upon their ionization constant (pKa) under the given pH of the pre-corneal tear film. The unionized compounds show higher lipophilicity enabling them to cross the outer epithelium. Apart from pKa, molecular weight, preservatives, presence of surfactants, vehicle, and osmolarity of the formulation are the other factors that determine the ocular pharmacokinetics of the drugs. Certain topical drug preservatives like benzalkonium chloride are reported to increase the ocular bioavailability of the topically applied drugs by disrupting the corneal membrane.

Most of the topically instilled drugs reach their Cmax in aqueous humor between 15 min to 2 hrs. Based on the frequency of drug administration hourly or 2 hourly, the levels can substantially be increased to reach pharmacologically accepted concentration in the cornea, conjunctiva, and aqueous humor. Pre-corneal drug residence time is largely dependent upon the bidirectional equilibration of drug levels reaching in between the cornea and tear film which slowly decrease along with time. This aspect gains much importance when trying to reach adequate antimicrobial drug levels after repeated drug instillation in case of corneal or conjunctival bacterial infections. Corneal nerves are very sensitive to non-specific stimulation, pH and osmolarity, the pre-corneal elimination of drugs which is in equilibrium with tissues can be accelerated in case if the lacrimal secretion is increased by the stimulation of sensory nerves of the cornea due to the nature of the drug component or formulation factors. Composition of topical formulation (drug vehicle) has also been reported to influence the topical penetration of drugs (Hardberger et al. 1975; McCarthy 1975).

3.2.3 *Compartment Models Used to Predict Ocular Kinetics of Drugs*

Compartment models are mathematical equations used to predict the drug disposition beyond the tissues/fluids in which the drug concentration is quantified. This information is required to understand the movement of drugs across various tissues and organs along with the elimination of drugs from the primary compartment. Usually for systemic pharmacokinetics, blood would serve as a central compartment from where the drug elimination predominately happens. In eye, anterior chamber administration of amikacin and chloramphenicol followed one compartmental model (Mayers et al. 1991). Following bolus administration of the hydrophobic immune-suppressant drug cyclosporine into the anterior chamber, its clearance from the aqueous humor was predicted by one-compartment model with the terminal half-life of 30–40 h, where as for the aqueous to tissue distribution into the cornea, a two-compartment model was employed (Oh et al. 1995).

Typically, when the analysis of drug partition into systemic and ocular tissues needs to be done, two-compartment model best suited the purpose considering that the multiple sites of tissue distribution is involved. Two-compartment modeling was successfully used by several investigators to predict the drug partitioning from tear film to ocular tissues for their effect as well as systemic absorption causing side effects. The advantage of timolol with thickening agent (gel) was compared with simple topical solution of timolol in rabbits. A two-compartment pharmacokinetic model was used to fit the aqueous humor level for determining the drainage (kd) and absorption rate constants (ka) in the pre-corneal area as well as the elimination rate constant (ke) of timolol in the aqueous humor. This study reported that gel has a longer retention time in eyes to improve ocular bioavailability and lesser systemic side effects (Chiang et al. 1996). Sasaki and coworkers (2000) predicted concentrations of tilisolol in the aqueous humor after instillation with CMC vehicle from the tear concentrations. Its ocular and systemic absorption was analyzed by a mathematical model including a diffusion process and a two-compartment model with first-order absorption, respectively. Similarly, a two-compartment model was successfully used for topically instilled gentamicin (Eljarrat-Binstock et al. 2004) and chlorhexidine gluconate (Xuguang et al. 2006). Presence of drug transporters is well recognized in the blood-ocular barriers; therefore, systemically administered drugs partitioning into the humors or tissues are subjected to their susceptibility for drug influx/efflux mechanisms. Systemically administered P-gp substrate (Rho-123) was best fitted with a three-compartment model and shifted to a two-compartment model upon its blocker administration in rabbits. This shift has increased intraocular penetration of compounds across blood-ocular barriers (Senthilkumari et al. 2008a, b). A three-compartment physiological-based pharmacokinetic (PK) model with a bidirectional transfer between the cornea and aqueous humor and a unidirectional transfer between the aqueous humor and iris-ciliary body was used to describe an antiglaucoma agent 1-ethyl-6-fluoro,1,2,3,4-tetrahydroquinoline in rabbits. Using its ED50 values derived from pressure-lowering activity (pharmacody-

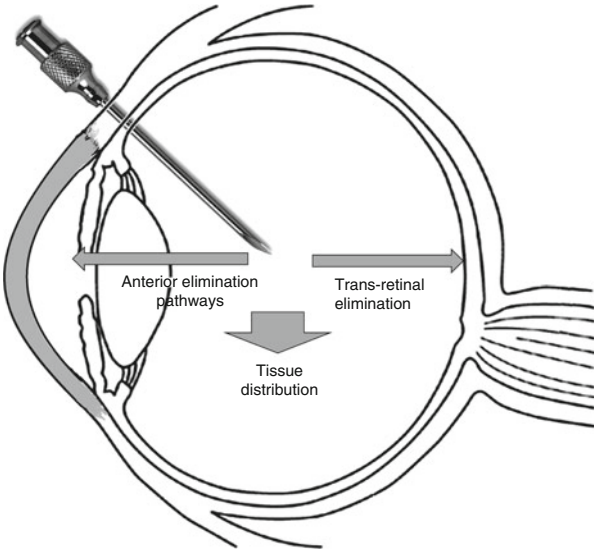

Fig. 3.2 Elimination pathways of intravitreally injected drugs

namic property), the PK-PD model was derived to explain pharmacodynamics of iris-ciliary body concentration time data (Pamulapati and Schoenwald 2012).

Intravitreally administered drugs reaching the systemic circulation is of concern for their toxicity (Fig. 3.2). Systemic pharmacokinetics of intravitreally administered ranibizumab in patients with retinal vein occlusion (RVO) or diabetic macular edema (DME) was predicted using one-compartment pharmacokinetics model with first-order absorption and elimination rate constants. This population kinetic study found that there is no difference among conditions like AMD, RVO, and DME as far as the systemic exposure of VEGF antibody is concerned (Zhang et al. 2014).

Subconjunctival injection of gentamicin was fitted into one compartmental model (van Rooyen et al. 1991). Antibacterial agents such as ciprofloxacin and fleroxacin (Miller et al. 1992) were also predicted with single-compartment model after direct intravitreal administration. A population pharmacokinetic metabolism model was used to describe the concentrations of ciprofloxacin in serum, aqueous and vitreous humor by a four-compartment PK linear model after oral administration.

Single-dose administration as eyedrop raises aqueous humor levels within a period of 30–60 min and mostly the levels fall within 4 h. Being underprivileged organ, eye lacks first-line immune defense mechanisms in the humors, antimicrobial drug levels above the MIC of microbe is required for their activity. Therefore, during corneal infections, hourly or 2 hourly instillations of antimicrobial agents are preferred. To estimate the appropriate drug schedule, pharmacokinetic simulations are helpful. Aqueous humor kinetics of single and multiple doses of topical, non-preserved voriconazole (VZ) were studied in human eyes comparing hourly versus 2 hourly instillations (Senthilkumari et al. 2010). Single-dose ocular kinetics of 1 % VZ resulted in a maximum mean aqueous concentration of 3.333 ± 1.61 μg/ml in

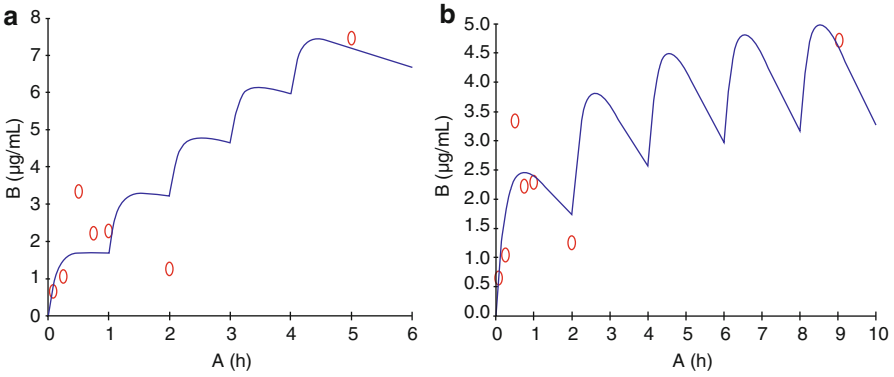

Fig. 3.3 PK simulation from our laboratory showing the predicted concentration of voriconazole in aqueous humor achieved hourly (A), 2 hourly (5 doses) instillation of voriconazole topical application in patients undergoing cataract surgery (*o* observed, -- predicted) (Senthilkumari et al. 2010)

30 min; multidose kinetic study revealed that hourly and bihourly dosing resulted in mean aqueous concentrations of 7.47 ± 2.14 µg/ml and 4.69 ± 2.7 µg/ml, respectively (Fig. 3.3).

Serial sampling of the humors of the eye is not a feasible task in human or animals while conducting ocular pharmacokinetic studies. Therefore, the model of collecting samples at various time points after the administration of drug is followed in all the studies except few studies indicating the worth of microdialysis for serial sampling in animals. Many of the ocular drugs were investigated in the literature for their intraocular penetration, but only few of them were interpreted with the help of pharmacokinetic models to explain their ocular disposition.

After a single topical instillation of tritiated clonidine HCl solution (0.2 %) at the volume of 30 µl into rabbit eyes, Chiang and Schoenwald (1986) evaluated clonidine ocular pharmacokinetics. Seven different tissues and plasma were excised and assayed for drug concentration over 180 min. They have also generated data at steady state levels of drug in cornea using an infusion assembly. The data were analysed by fitting them into physiological and a classical diffusion pharmacokinetic models. This study reported that the physiological model parameters were fit to the topical infusion data and showed good agreement between the predicted and experimental data.

3.2.4 Drug Interaction in Topically Applied Drugs

More than one eyedrops are often required to be administered together at times. In such conditions, chemical incompatibility has been observed which is causing one of them to precipitate in the lower fornix. This is a potential cause for the reduction of pre-corneal drug availability of the topically applied drugs. Ciprofloxacin

hydrochloride deposition on the lower fornix of the eye due to its pH incompatibility has been claimed as the advantage of pre-corneal deposits as drug depots "ciprofloxacin HCl precipitates." However, redistribution or dissolution of these deposits back to increase tear film concentrations have not been proved. Ocular pharmacokinetics of the topically applied drug also gets altered by coadministered drugs when they are competing for the same transport mechanism (Nirmal et al. 2013a). Coadministration of local anesthetics and antimuscarinic agents might increase ocular bioavailability of the drugs by decreasing tear film secretion. This interaction so far is attributed to the decrease in tear film secretion; however, other interactions on the corneal transport mechanism require further studies.

3.2.5 Ocular Drug Levels after Systemic Administration

Ocular pharmacokinetics of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was investigated using its levels in vitreous and aqueous humors after its systemic administration in rabbits. This study compared systemic, topical, and subconjunctival administrations and suggested that topical application of BCNU is the best and subconjunctival injection the second best route for treating an iris tumor and that intravenous injection is the best route for choroidal and retinal tumors (Ueno et al. 1982).

Aqueous humor penetration of amikacin was assessed in the anterior chamber of the human eyes using radioimmunoassay. This study reported that bactericidal concentrations of amikacin were not achieved by topical or intravenous administration. Subconjunctival injection also did not produce consistent bactericidal concentration of amikacin in the aqueous humor. This study has also reported that iris pigment binding as one of the factor responsible for the poor levels reached (Eiferman and Stagner 1982). Several clinical studies showed that inadequate penetration of antimicrobial agents into the eye after their systemic administration as a limitation for ocular infections (Velpandian 2009). Only after multiple doses orally administered fluoroquinolones like ciprofloxacin reached significant levels in the vitreous above the MIC for most of the ocular pathogenic organisms (Keren et al. 1991). Inadequate penetration of different classes of drugs into non-inflammed eyes after systemic administration has been well documented by many investigations.

3.2.6 Factors Affecting Ocular Penetration of Drugs

Aqueous ophthalmic drug solutions typically exhibit low bioavailability due to various loss processes such as drainage, tear turnover, nonproductive absorption, and protein binding. Suspensions may improve bioavailability, but because of a short residence time and a low corneal permeability rate constant, the dissolution rate of the drug and its intrinsic solubility must be considered.

Topically applied hydrophobic compounds like amphotericin B showed undetectable levels of drug after single application; however, after repeated application,

therapeutic levels reached the cornea in the normal eyes of rabbits. In inflamed eyes the levels reached high quickly but fell rapidly (O'Day et al. 1986). Corneal inflammation that induced increased drug penetration across the cornea is not well documented in the literature due to the difficulties encountered in sampling aqueous humor from patients undergoing ocular surgeries.

3.2.7 Ocular Drug Metabolism

Although the liver is still the major organ involved in the biotransformation of drugs, drug metabolism in the eye is often pursued as a avenue for the development of designer drugs. The corneal epithelium is the source of amidases and esterases which favors the optimization of dosage forms using the concept of prodrugs. Latanoprost and dipivefrin are the best examples for the prodrugs to get into their active form after the metabolism in the cornea. In the cornea, the presence of aminopeptidases and other peptidases has also been reported, and their involvements in the metabolism of drugs are confirmed (Lee et al. 1986). While studying the ocular pharmacokinetics of topically applied phenylephrine, Antoine et al. (1984) reported that the corneal epithelium is responsible for the metabolic degradation of phenylephrine which occurred following its topical instillation. While working with levobunolol for topical antiglaucoma treatment, Tang-Liu and coworkers (1987) demonstrated that the major sites of ocular metabolism were the corneal epithelium and the iris-ciliary body. On passage across the cornea, 4.7 % of topically applied levobunolol dose was biotransformed to an active metabolite dihydrolevobunolol and subsequently became bioavailable to intraocular tissues. Another 12 % of the topical levobunolol dose entered the systemic circulation as metabolite after presystemic biotransformation. Nakamura et al. (2005) examined the expression levels of the different conjugation enzymes, sulfotransferases, UDP-glucuronosyl transferases (UGTs), and glutathione S-transferases (GSTs), in ocular tissues. In 5-week-old animals, the CYP genes, CYP2B2 and CYP3A1, were abundantly expressed in the lens, with higher CYP1A1 expression detectable in the extra-lenticular tissues, of both genders. They have also reported that in general, the expression levels of the CYPs and sulfotransferases declined with age, whereas the levels of the UGTs and GSTs increased. These results demonstrate that the expression profiles of drug-metabolizing enymes show both region- and age-specific patterns in rat ocular tissues. Presence of various metabolizing enzymes and their physiological function has been targeted for ocular drug delivery (Duvvuri et al. 2004).

3.2.8 Elimination Pathways of the Drugs

A major pathway for systemic absorption of topically applied drug has been reported to be through the walls of the gastrointestinal tract (Anderson 1980). Schmitt et al. (1980) evaluated the penetration of radiolabeled timolol into the rabbit eye after

topical instillation and after intravenous injection in animals. This study reported that the levels of radioactivity were considerably greater in ocular tissues after instillation as compared with intravenous injection, whereas in extraocular tissues, the levels were similar after both routes of administration. This study has also reported that in ocular instillation, only unchanged timolol was present in the aqueous humor, whereas both timolol and metabolites were present in the serum. After intravenous administration, timolol was rapidly metabolized and metabolites appeared in the serum and aqueous humor. Pharmacokinetic studies conducted after the intravitreal injection of drugs revealed that two predominating pathways are involved in the clearance of drugs. Most of the drugs are cleared through posterior elimination pathway through retinal blood vessels Drugs like aminoglycoside antibiotics follow anterior pathway through aqueous humor and iris (Barza et al. 1983; Gupta et al. 2000; Nirmal et al. 2012).

3.2.9 Functional Importance of Blood-Ocular Barriers Affecting Pharmacokinetics

Drug penetration across blood-ocular barriers is now well recognized; therefore, beyond pharmaceutical parameters, their susceptibility for various transporters is expected to govern their pharmacokinetics. Probenecid (Benemid) was the agent first explored to elevate serum penicillin concentrations (Burnell and Kirby 1951). The interest to evaluate the impact on the concentration of penicillin derivatives in ocular fluids (Barza et al. 1973; Salminen 1978). However, these studies showed that administration of probenecid had an enhancing effect on ocular cloxacillin concentration allowing improved drug diffusion into the eye by means of an elevated plasma concentration and had no specific ocular effect. In the subsequent studies after the intravitreal administration of carbenicillin, concomitant intraperitoneal dosing of probenecid prolonged the vitreal half-life of carbenicillin and showed that beta-lactam antibiotics are eliminated via the retinal route. Ever since, observations and curiosities among the researchers led to better fundamental understanding regarding the involvement of drug transporters in blood-ocular barriers. Using retinal capillary endothelial cell lines presence and function of various transporters were explored (Hosoya and Tomi 2005; Mannermaa et al. 2006). Subsequently, functional importance of such transporters for the penetration of xenobiotics reported in animals (Senthilkumari et al. 2009; Nirmal et al 2012; Gunda et al. 2006)

Although, the presence of transporter proteins in the blood-ocular barriers has been well documented, their extent and role affecting pharmacological action have come to light after the systematic explorations in the last decade. In these controlled experiments, probes were used as a substrate and their pharmacokinetic modulation was assessed after blocking the functions of transporters with respective agents.

Functional role of P-gp and ocular tissue distribution of intravitreally injected rhodamine 123 (Rho-123) was evaluated in the presence of P-gp-specific blocker (GF 120918) in normal as well as rifampicin-fed rabbits using microdialysis and

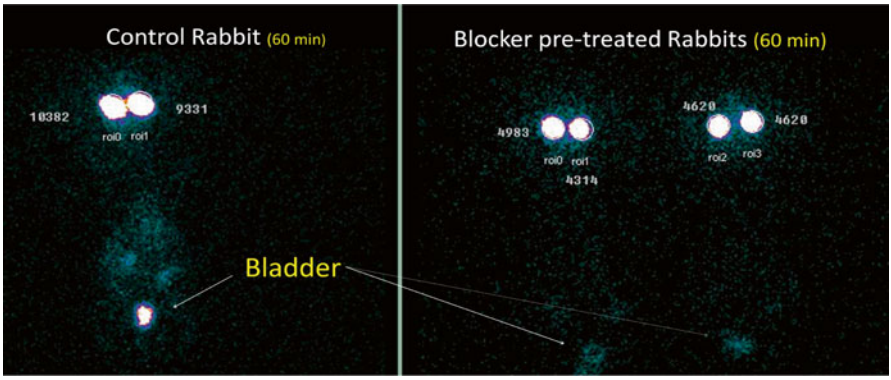

Fig. 3.4 Static planar gamma camera image of rabbits following intravitreal injection of ^{99m}Tc-ofloxacin in rabbits. Note: Control rabbit bladder showing higher radioactivity

direct sampling technique. This study revealed that intravenously injected blocker significantly altered the ocular disposition of intravitreally injected P-gp substrate. Rifampicin pretreatment did not upregulate P-gp transporters of the retina to the extent to affect the intravitreal kinetics of Rho-123 significantly (Senthilkumari et al. 2008a). The impact of P-glycoprotein (P-gp) blockade on the intravenous (i.v.) pharmacokinetics of Rho-123 and the subsequent effect on its disposition in ocular and non-ocular tissues were studied using rabbits. This study concluded that increasing the ocular concentration of systemically given drugs may not be possible with the degree of P-gp blockade achieved when using GF120918 (P-gp blocker) at the studied concentration after intravenous administration (Senthilkumari et al. 2008b). The effect of P-glycoprotein modulation at blood-ocular barriers using gamma scintigraphy in rabbits is shown in Fig. 3.4. This confirmed the involvement of P-glycoprotein in the intraocular disposition of susceptible drugs (Senthilkumari et al. 2009).

Although aminoglycoside antibiotics were reported to be cleared through the anterior route (Barza et al. 1983), while studying OCT transporters, Nirmal et al. (2013b) concluded that the clearance of organic cation transporter (OCT) substrates favors the anterior elimination pathway. The functional importance of (OCT) on the ocular disposition of intravitreally injected substrate tetraethyl ammonium (TEA) was assessed in rabbits (Nirmal et al. 2013b); this study concluded that intravitreally injected OCT substrates may follow an anterior elimination pathway and prolonged residence time in the vitreous humor. This study showed that OCT may not be active from vitreous to blood route in the blood-retinal barrier.

The potential pharmacokinetic role of organic cation transporters in modulating the trans-corneal penetration of its substrates administered topically has been studied in rabbits. This study concluded that OCT is functionally active in the cornea causing uptake of their substrates from tear to the aqueous humor. When administering their substrates/blockers topically, both may be competing for OCT for their uptake across the cornea, thereby decreasing the corneal penetration. Hence, OCT can have a potential pharmacokinetic role in modulating the ocular bioavailability

of their substrates which are used topically for ocular therapeutics (Nirmal et al. 2013b). The role of organic cation transporters was studied in the ocular disposition of its intravenously injected substrate in rabbits by quantifying the levels of its substrate in the presence and absence of blockers. This study revealed that in most of the tissues, OCTs are functionally present from apical to basolateral. The gene expression studies also showed the presence of OCT1, OCTN1, and OCTN2 in various ocular tissues studied. This study suggested that OCTs are functionally active in blood-ocular barriers and involved in the transport of its substrate from blood to vitreous humor (Nirmal et al. 2013a). Moreover, this study also revealed the precorneal availability of OCT substrate through lacrimal secretion indicating the possibility of utilizing them for the delivery of drug to the tear film through systemic route (Nirmal et al. 2010). Apart from OCT, OAT, and P-gp transporters, PEPT transporters have been used for the delivery of stable dipeptide prodrugs for improved absorption of acyclovir (Talluri et al. 2008).

Most of the drugs used in ophthalmic therapeutics are developed for systemic diseases, which are subsequently included for ocular use after toxicity studies. Developing any drug with the understanding of the constraints of the eye was not a popular strategy followed. Much of the emphasis was made on pharmaceutical solutions rather than relevant molecular parameters. The cornea is a live tissue that behaves much beyond simple biomatrix; therefore, in vitro studies may not be having much relevance in the in vivo scenario. To enable in silico screening of drugs, techniques like cassette dosing are being developed to assess the possibility of enhanced topical penetration with less number of animals (Sharma et al. 2011). Along with the increasing knowledge about the barrier functions, it is clear that the in-depth knowledge of the molecular properties like drug transport susceptibility, and interaction of drugs with their physiological/pathological targets are expected to govern ocular pharmacokinetics and toxicity of drugs in the future.

3.3 Conclusion

In the current state of knowledge, it is evident that developing ocular specific drug to meet the target site concentration with reduced systemic exposure is a possible reality. Screening drugs with better ocular pharmacokinetics for their projected use in ophthalmic therapeutics is the rationalized approach for ocular drug development.

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