Drug transport by the blood-aqueous humor barrier of the eye

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Abbreviations: Apical sodium dependent bile salt transporter, ASBT; Breast cancer resistant protein, BCRP; Multidrug and toxin extrusion transporter, MATE; Multidrug-resistance associated protein, MRP; Organic anion transporter (OAT); Organic anion transporting polypeptide, OATP; Organic cation transporter, OCT; Organic cation transporter novel, OCTN; Peptide transporter, PEPT; P-glycoprotein, Pgp; Sodium dicarboxylate cotransporter, NaDC; Sodium taurocholate cotransporter, NTCP
Abstract

The ocular barriers (cornea, blood-retinal barrier and blood-aqueous humor barrier) make treating eye diseases with therapeutic drugs challenging. The tight capillary endothelium of the iris and the ciliary body epithelium form the blood-aqueous humor barrier. The iris and ciliary body (iris-ciliary body) express a variety of drug transporters in the ATP-binding cassette (ABC) and solute carrier (SLC) families. Drug transporters in the ABC family that are present in the iris-ciliary body include P-glycoprotein (Pgp), breast cancer resistant protein (BCRP) and several multidrug resistance-associated proteins (MRPs). Drug transporters in the SLC family that are present include organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), bile acid transporters (ASBT and NTCP), organic cation transporters (OCTs, OCTNs and MATEs) and peptide transporters (PEPTs). Freshly dissected iris-ciliary body preparations actively accumulate a variety of substrates of SLC drug transporters that are expressed in the tissue. The ciliary body in vitro supports active transport in the aqueous humor-to-blood direction of several substrates of OATs and MRPs, consistent with the subcellular localization of these transporters in the ciliary body epithelium. In vivo data suggest that drug transporters in the iris-ciliary body reduce the permeation of drugs in the direction of blood-to-aqueous humor, thereby reducing ocular drug bioavailability, but also, are involved in active drug elimination from the aqueous humor. A better understanding of the influence on pharmacokinetics of drug transporters in the blood-aqueous humor barrier should help improve drug delivery and efficacy in the eye.
Introduction

The eye is a pharmacological sanctuary, making it challenging to treat many ocular diseases with therapeutic drugs. This is in part due to the presence of the ocular barriers – cornea, blood-retinal barrier (inner and outer) and blood-aqueous humor barrier (iris-ciliary body). The resilience of the eye to drug exposure is evident from the number of ocular drug delivery methods in development and use. Topical, systemic, periocular and intraocular are the main routes for drug delivery to the eye (Geroski and Edelhauser, 2000). Therapeutic concentrations can be achieved with both topical and systemic administration, but often requires the use of relatively high doses and/or drugs with sufficient hydrophobicity to efficiently cross the ocular barriers and cornea (Barar, et al., 2008; Gaudana, et al., 2010). Although topical eye drop administration is the least invasive, only a small fraction of the administered dose reaches the eye interior (Maurice DM and Mishima S, 1984). Systemic administration can be an effective delivery method, but the high doses often needed to achieve effective intraocular concentrations can cause systemic toxicity. For example, intravenous administration of melphalan can be effective at treating retinoblastoma, but the doses required to achieve a therapeutic response are associated with severe bone marrow toxicity (Shields and Shields, 2010). Periocular and intraocular administration are used to avoid poor drug penetration across the ocular barriers. However, once inside the elimination rate of select drugs is rapid, often necessitating the use of high doses, repeated administration or extended release systems to counteract short drug half-lives (Geroski and Edelhauser, 2000; Yasukawa, et al., 2005). The poor intraocular penetration of drugs administered outside of the eye and their rapid elimination once inside suggests that active mechanisms contribute, along with passive processes (passive diffusion across epithelia and aqueous outflow), to poor ocular drug delivery. Indeed, there is
considerable evidence that drug transporters expressed in the blood-aqueous humor barrier, blood-retinal barrier and cornea function to reduce the intraocular bioavailability of systemically and topically administered drugs, and can facilitate their elimination from both the aqueous and vitreous humor. Several previous reviews have discussed in detail drug transport by the blood-retinal barrier and cornea (Mannermaa, et al., 2006; Nakano, et al., 2014; Tomi and Hosoya, 2010; Chemuturi and Yanez, 2013; Jordan and Ruiz-Moreno, 2013; Hosoya and Tachikawa, 2009), whereas little attention has been given to the blood-aqueous humor barrier (i.e., iris-ciliary body).

Although there are numerous drug transporters that interact with organic electrolytes that display a net negative charge (organic anions), a net positive charge (organic cations), or both negative and positive charges (zwitterions) at physiological pH, most of our understanding of drug transport by the iris-ciliary body come from studies using organic anions as ligands – i.e., substrates and/or inhibitors. Research in the 1960’s and 1970’s by Becker (Becker, 1960), Forbes and Becker (Forbes and Becker, 1960), and Bárány (Barany, 1972; Barany, 1973b; Barany, 1973a; Barany, 1974; Barany, 1975; Barany, 1976) show that organic anion transport by the iris-ciliary body has properties similar to those found in liver and kidney – the two major excretory organs for drugs, and determinants of systemic pharmacokinetics. Even back then, before the molecular identity of drug transporters were known, it was speculated that these active transport mechanisms in the iris-ciliary body were important for ‘scavenging’ from the aqueous and vitreous humor potential xenobiotic and endobiotic toxins that could be deleterious to vision (Barany, 1976). This review covers 1) the tissue-, cellular and subcellular expression of drug transporters in the iris-ciliary body, 2) in vitro evidence for drug transport activity in the iris-ciliary body (with a focus on organic anions), and 3) in vivo evidence for the
involvement of active transport in influencing intraocular drug concentrations, with a likely contribution from the blood-aqueous humor barrier.

**Drug transporters**

Drug transporters support flux across plasma membranes of relatively small (<1000 mol wt) organic molecules (organic anions, cations and zwitterions) that are structurally diverse. Included within this class of chemicals are pharmacologically and toxicologically relevant xenobiotics along with endogenous chemicals of physiological importance (endobiotics) (Klaassen and Aleksunes, 2010). Due to their charge at physiological pH, efficient movement of organic electrolytes across plasma membranes requires facilitated transport. Drug transporters are expressed in a variety of barrier epithelia/endothelia, such as the intestinal epithelium, liver hepatocytes, kidney tubules and brain capillary endothelium, where they influence tissue drug concentrations, i.e., pharmacokinetics (Klaassen and Aleksunes, 2010). Drug transporters are contained within two distinct families, the solute carrier (SLC) family and the ATP-binding cassette (ABC) family. Transporters in the ABC family hydrolyze ATP to facilitate efflux of their substrates out of cells. The multidrug resistance associated proteins (MRPs), breast cancer resistant protein (BCRP) and P-glycoprotein (Pgp) are examples of drug transporters contained within the ABC family. SLC drug transporters use a variety of energetic mechanisms to support solute flux, including Na⁺-dependent co-transport, exchange and electrogenic facilitated diffusion, and can facilitate cellular uptake or efflux depending on the electrochemical driving forces. However, most of the SLC drug transporters discussed here support cellular accumulation of their substrates in other tissues – the MATEs are an exception. Notable examples of SLC drug transporters are the organic anion transporting polypeptides (OATPs), organic anion transporters (OATs), organic cation transporters (OCTs) and multidrug and toxin
extrusion transporters (MATEs) – although there are others. A key feature of drug transporters is their broad and sometimes overlapping ligand selectivity. SLC and ABC drug transporters likely evolved with increasing physiological complexity to provide protection from exposure to an increasing diversity of xenobiotics (Eraly, et al., 2004). Indeed, many of the SLC and ABC drug transporters have arisen by gene duplication and display unique but also overlapping ligand selectivity (Eraly, et al., 2004; Moitra and Dean, 2011). Within the ocular barriers drug transporters likely function to protect tissues that are important for vision from exposure to potentially toxic xenobiotics and endobiotics, especially the avascular lens epithelium and cornea. Importantly, many of the drug classes used to treat ocular disease contain small molecule drugs that are substrates of drug transporters, such as antibiotics, antivirals, anti-inflammatory drugs, α-adrenergic receptor agonists, prostaglandin analogs and antimetabolites.

The blood-aqueous humor barrier

The blood-aqueous humor barrier consists of the ciliary body epithelium and the tight capillary endothelium of the iris (Cunha-Vaz, 1979). The ciliary body epithelium is responsible for secretion of aqueous humor into the posterior chamber of the eye. The ciliary body extends posteriorly from the iris root to the retina, forming a ring around the globe. The ciliary body has two structurally distinct segments, the pars plicata and pars plana. The pars plicata is located anteriorly and is characterized by extensive villi-like foldings called ciliary processes. The pars plana is scalloped and is positioned between the pars plicata and the retina. The ciliary body is highly vascularized by the choroidal capillaries, which are fenestrated and leaky (Friedenwald and Stiehler, 1938; Stewart and Tuor, 1994). The iridial capillaries are continuous and contain tight junctions, but have a higher permeability than inner retinal capillaries (i.e., the inner blood-retinal barrier) (Alm, 1992). The ciliary body is a bilayer comprised of two distinct cell layers –
a pigmented cell layer and a non-pigmented cell layer. The pigmented and non-pigmented cells are coupled via gap junctions (Shin, et al., 1996; Coffey, et al., 2002). The pigmented cells contact the choroidal blood supply, whereas the basolateral membrane of the non-pigmented epithelium contacts aqueous humor. Tight junctions are present in the non-pigmented epithelial cells, but not in the pigmented cells (Sonsino, et al., 2002). Thus, the physical barrier to drug movement across the ciliary body is the non-pigmented epithelium.

Expression of ABC transporters in iris-ciliary body

Several studies have examined mRNA (RT-PCR, qPCR or microarray) expression of ABC drug transporters in isolated human iris-ciliary body (Zhang, et al., 2008; Chen, et al., 2013), or in preparations containing only human iris or ciliary body that were isolated separately by microdissection (Pelis, et al., 2009; Dahlin, et al., 2013; Lee, et al., 2015) – Table 1 summarizes the findings from these studies. In general, the studies are in good agreement with each other. The majority of the studies observed mRNA for MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, MRP7, BCRP and Pgp in either ciliary body or iris-ciliary body preparations, with only a few discrepancies. For example, Chen et al. (Chen, et al., 2013) failed to observe mRNA for MRP3 in iris-ciliary body while Zhang et al. (Zhang, et al., 2008) did not detect MRP2 mRNA in iris-ciliary body. Of the ABC transporter genes examined by Dahlin et al. (Dahlin, et al., 2013), MRP5 and Pgp (MRP5>Pgp) showed the highest expression level in the human ciliary body (Dahlin, et al., 2013). At the protein level (immunoblotting), MRP1, MRP2, MRP3, MRP4, MRP6, MRP7 and Pgp were detected in human iris-ciliary body (Chen, et al., 2013), and MRP1, MRP2, MRP4, BCRP and Pgp were detected in human ciliary body (Pelis, et al., 2009) (Figure 1). While Pelis et al. (Pelis, et al., 2009) detected BCRP protein in human ciliary body from a single donor by immunoblotting, another study failed to detect BCRP protein
expression in iris-ciliary body from multiple donors (Chen, et al., 2013). MRP2 localizes to the apical membrane of non-pigmented epithelial cells of the human pars plana and plicata (Pelis, et al., 2009), whereas MRP4 is located in the basolateral membrane of pigmented cells (Lee, et al., 2015) (Figure 2). Both transporters are speculated to play a role in effluxing drugs from ciliary epithelial cells into the choroid. In two independent studies, mRNA for MRP1, MRP3, MRP4, MRP5, MRP6, MRP7 and Pgp were detected in human iris, whereas BCRP was low to undetectable (Dahlin, et al., 2013; Lee, et al., 2015) (Table 1). Of the ABC transporter genes examined in the iris by Dahlin et al. (Dahlin, et al., 2013), Pgp and MRP5 (Pgp>MRP5) were the most highly expressed. Although Dahlin et al (Dahlin, et al., 2013) detected MRP2 mRNA in human iris, Lee et al. (Lee, et al., 2015) did not. Within the human iris, protein for MRP1, MRP2, MRP4 and BCRP were observed (Pelis, et al., 2009) (Figure 1). Pgp has been localized to iridial vessels by immunohistochemistry (Schlingemann, et al., 1998; Holash and Stewart, 1993) (Figure 2).

**Expression of SLC transporters in the iris-ciliary body**

The mRNA expression of numerous transporters in the SLC family have been examined in the iris-ciliary body, ciliary body or iris of humans (Table 2). Most of the SLC transporters listed are cellular uptake transporters. In some cases there is a discrepancy between the studies regarding whether mRNA for the transporters are expressed in the ciliary body. Regardless, from Table 2 it is apparent that both the ciliary body and iris express many of the SLC transporters known to influence pharmacokinetics, and some transporters with important physiological roles, such as transporters of bile acid acids (ASBT and NTCP), dicarboxylates (NaDC3), peptides (PEPT1 and PEPT2), ergothioneine (OCTN1) and carnitine (OCTN2).
Several of the transporters listed in Table 2 have been examined at the protein level in human ciliary body by immunoblotting and/or immunohistochemistry. Protein for OATP1A2, OATP1C1, OATP2B1, OATP3A1 and OATP4A1 were detected in human ciliary body extracts by immunoblotting (Gao, et al., 2005) (Figure 1). Kraft et al. (Kraft, et al., 2010) also detected mRNA for OATP2A1 and OATP2B1 by qPCR in human ciliary body as well as iris – albeit, the expression of OATP2A1 in iris was near undetectable in their study. Gao et al. (Gao, et al., 2005) demonstrated expression of OATP2B1, OATP3A1 and OATP4A1 protein in the basolateral membrane of non-pigmented epithelial cells of the pars plicata, while OATP1A2 and OATP1C1 were undetectable in the pars plicata (Figure 1). At odds with the study by Gao et al. (Gao, et al., 2005), another study observed expression of OATP2B1 in basolateral membranes of both non-pigmented cells as well as pigmented cells of the pars plicata (Kraft, et al., 2010) (Figure 2). Thus, it is not clear based on this discrepancy whether OATP2B1 is present in the basolateral membrane of pigmented cells of the pars plicata. OATP2A1 localizes to basolateral membranes of pigmented cells and non-pigmented cells of the pars plicata (Kraft, et al., 2010) (Figure 2). In the pars plana, OATP1A2 and OATP2B1 are restricted to the basolateral membrane of non-pigmented epithelial cells, and OATP1C1, OATP3A1 and OATP4A1 are present in basolateral membranes of non-pigmented epithelial cells as well as pigmented cells (Gao, et al., 2005) (Figure 2). Both OATP2A1 and OATP2B1 also immunolocalize to iridial vessels (Kraft, et al., 2010) (Figure 2). Importantly, prostaglandins are well-known substrates of several OATPs (Hagenbuch and Stieger, 2013), and prostaglandin analogs, such as unoprostone and latanoprost are anti-glaucoma drugs. The metabolite of unoprostone (unoprostone carboxylate) is a substrate of OATP1A2, OATP2B1 and OATP4A1 (Gao, et al., 2005), and latanoprost is a substrate of OATP2B1 and OATP2A1 (Kraft, et al., 2010), suggesting that
OATPs in the ciliary body may influence the pharmacological action of prostaglandin analogs used to treat glaucoma.

In addition to several OATPs, protein for the renal organic anion transporters OAT1 and OAT3 were detected in ciliary body from four different human donors by immunoblotting (Lee, et al., 2015) (Figure 1). Within the kidney, both OAT1 and OAT3 use energy in the outwardly-directed α-ketoglutarate (Kreb’s cycle intermediate) gradient to facilitate cellular substrate uptake via an anion exchange mechanism, and this gradient is maintained in part by the Na-dependent dicarboxylate cotransporter 3 (NaDC3) (Pelis and Wright, 2011). Accordingly, NaDC3 protein was detected in ciliary body from four human donors by immunoblotting (Lee, et al., 2015) (Figure 1). Within the pars plicata of the human ciliary body both OAT1 and OAT3 are present in the basolateral membrane of non-pigmented epithelial cells, along with Na,K-ATPase (Figure 2) (Lee, et al., 2015). Although the subcellular localization of NaDC3 in ciliary body is unknown, Nadc3 mRNA is present in non-pigmented epithelial cells of mice, as shown by in situ hybridization (George, et al., 2004).

While the previous discussion of drug transporter expression in the blood-aqueous humor barrier comes from studies that primarily used human tissues, in vitro and in vivo evidence discussed below comes mostly from studies with other species (rabbit, monkey and bovine). It is important to point out that species differences in drug transport exist. For example, whereas humans have a single Pgp gene (ABCB1), rodents have two genes corresponding to Pgp (Abcb1a and Abcb1b, also referred to as Mdr1a and Mdr1b). Regardless, many of the drug transporter orthologs discussed in this review have ligand selectivities that are conserved across species – example, Mdr1a and Mdr1b share many of the same ligands that interact with human Pgp.
In vitro evidence for drug transporter activity in iris-ciliary body

In vitro data using fresh preparations of iris-ciliary body, or ciliary body devoid of iris indicate that drug transporters, in particular, organic anion transporters, are active in the iris-ciliary body. The renocystographic agent iodohippurate and the cholecystographic agent iodipamide, which actively accumulate in kidney tubules (Barany, 1972) and liver hepatocytes (Joppen, et al., 1985), respectively, are actively taken up by the ciliary body (ciliary processes) from a variety of species (Barany, 1972). Freshly excised segments of rabbit iris-ciliary body accumulate iodopyracet to ~15-fold above the bath iodopyracet concentration, and accumulation is saturable, dependent on oxidative metabolism, temperature-sensitive, and inhibited by several organic anions that are ligands of organic anion transporters, including para-aminohippurate, chlorophenol red, penicillin and probenecid (Becker, 1960). Interestingly, iodopyracet is actively secreted by renal tubules in a probenecid-sensitive manner, an active process likely mediated by the classical renal organic anion secretory system, which involves the uptake transporters OAT1 and/or OAT3 (Russel, et al., 1989). Chlorophenol red, which is secreted by organic anion transporters in renal tubules, actively accumulates in rabbit ciliary body segments that are devoid of iris (Sugiki, et al., 1961). That is, accumulation is metabolic- and temperature-dependent, and inhibited by iodopyracet, probenecid and penicillin (Sugiki, et al., 1961). Also, the well-established substrate of the renal organic anion secretory system and OAT1 substrate para-aminohippurate accumulates in the monkey ciliary body in vitro, and independently in the iris as well (Stone, 1979). Para-aminohippurate accumulation by the monkey ciliary body and iris is saturable, and active transport is inhibited by metabolic poisons, Na,K-ATPase inhibition (ouabain), iodopyracet and probenecid (Stone, 1979). Several bile acids, including cholate, glycocholate, deoxycholate and chenodeoxycholate show significant temperature-dependent
accumulation by rabbit iris-ciliary body preparations, and accumulation is sensitive to iodipamide (Barany, 1975). Consistent with these data, the ciliary body and iris express mRNA for several bile acid uptake transporters, including ASBT, NTCP and several OATPs (Table 2). Prostaglandin F2α accumulation by rabbit iris-ciliary body is saturable, and is inhibited by metabolic poisons and a variety of organic anions, including probenecid, bromocresol green, iodipamide and indomethacin, but is insensitive to the prototypic OAT1 substrate para-aminohippurate (Bito, et al., 1976). As indicated previously, prostaglandins are well-established substrates of several OATP uptake transporters (Hagenbuch and Stieger, 2013). The studies with fresh preparations of iris-ciliary body show that the tissue(s) support active drug transport. However, given the heterogeneity of cell types (hematopoietic, non-pigmented cells and pigmented cells) expressed in this preparation, it is not possible from these studies to distinguish the cellular source of the activity.

The aforementioned studies indicate that the iris-ciliary body expresses a variety of drug transporters in the ABC and SLC families, and that the tissue can actively accumulate known substrates of several SLC drug uptake transporters, but can the tissue support active transepithelial transport of drugs? Two studies have in fact shown that the ciliary body mounted in Ussing chambers can preferentially transport organic anions in the aqueous humor-to-blood direction. The strength of the Ussing chamber technique is that by eliminating the chemical (identical saline on both sides of the tissue) and electrical (voltage-clamped conditions) gradient across the tissue preparation, it allows for the determination of ‘active’ transepithelial transport of solutes. The organic anions tested were 6-carboxyfluorescein, para-aminohippurate and estrone-3-sulfate, all of which are substrates of OAT1 and/or OAT3, and that are secreted by renal proximal tubules (Pesis and Renfro, 2004). Kondo et al. (Kondo and Araie, 1994) showed
a transport flux ratio (aqueous humor-to-blood flux/blood-to-aqueous humor flux) for 6-carboxyfluorescein (10 μM) of ~5 across the rabbit ciliary body. The blood-to-aqueous humor flux of 6-carboxyfluorescein occurs via passive diffusion, whereas the aqueous humor-to-blood flux is saturable, with an apparent Michaelis constant of 28 μM (Kondo and Araie, 1994). Active aqueous humor-to-blood flux of 6-carboxyfluorescein is inhibited by ouabain (Na⁺,K⁺-ATPase inhibition), low bath [Na⁺], metabolic inhibition (2,4-dinitrophenol), low temperature, probenecid, iodipamide and hippurate (Kondo and Araie, 1994). OAT1 and OAT3 are Na⁺-independent anion exchangers but cellular substrate uptake by the transporters is indirectly dependent on the Na⁺-gradient for generation of the α-ketoglutarate gradient across the plasma membrane, which in part is established by Na⁺,K⁺-ATPase and by Na⁺-dependent dicarboxylate cotransporters. The Na⁺-dependence of active 6-carboxyfluorescein transport by the ciliary body suggests coordination of Na⁺-dicarboxylate cotransport activity (likely NaDC3) and OATs in active organic anion transport by the ciliary body epithelium. Consistent with the active transport of 6-carboxyfluorescein in the aqueous humor-to-blood direction across the ciliary body in Ussing chambers, the elimination rate of 6-carboxyfluorescein from the perfused bovine eye in vitro is slowed ~5-fold by the OAT1 and OAT3 inhibitor, novobiocin, when it is co-administered to the aqueous humor (Lee, et al., 2015). The perfused bovine eye preparation allows for administration of drugs directly into the aqueous humor, and for measurement of drug appearance in the venous effluent due to the combined influence of passive aqueous humor outflow and transport across the blood-aqueous humor barrier.

The flux ratio for para-aminohippurate transport (para-aminohippurate bath concentration, 1 mM) and estrone-3-sulfate transport (estrone-3-sulfate bath concentration, 5 μM) across the bovine ciliary body in Ussing chambers was 5.3-fold and 3.5-fold, respectively –
indicating preferential transport in the blood-to-aqueous humor direction (Lee, et al., 2015). Net active transport of para-aminohippurate across the bovine ciliary body in the aqueous humor-to-blood direction is inhibited by probenecid and novobiocin, but also by the MRP inhibitor MK571 (Lee, et al., 2015). The sensitivity of transepithelial organic anion transport to a variety of inhibitors, the expression of OAT1 and OAT3 in the basolateral membrane of non-pigmented epithelial cells, MRP2 in apical membrane of non-pigmented epithelial cells, and MRP4 in basolateral membrane of pigmented cells (Figure 2), is in agreement with the active transport by the ciliary body of para-aminohippurate, 6-carboxyfluorescein and estrone-3-sulfate in the direction of elimination from the aqueous humor.

**In vivo evidence for drug transporter function in the eye**

In vivo studies using either systemic (intravenous or oral) or intraocular drug administration provide evidence that drug transporters can reduce drug concentrations in the eye either by limiting drug penetration across the ocular barriers (i.e., a functional barrier) and/or by facilitating drug elimination once inside. The Pgp substrate cyclosporine A administered orally to humans (BenEzra, et al., 1990), rabbits (BenEzra and Maftzir, 1990a) and rats (BenEzra and Maftzir, 1990b) at therapeutic doses is not detected in the aqueous humor or vitreous, except when the ocular barriers are disrupted by inflammation, suggesting that Pgp-mediated efflux plays an important role in drug absorption across the ocular barriers following systemic administration. Indeed, following intravenous administration of the Pgp substrates quinidine, digoxin and verapamil, their aqueous humor uptake index values (concentration of drug in aqueous humor/concentration of drug in blood) are lower than predicted based on their log $D_{7.4}$ values, but are increased in the presence of Pgp inhibitors (Toda, et al., 2011)(Fujii, et al., 2014). Kajikawa et al (Kajikawa, et al., 2000) showed that the aqueous humor clearance of the P-gp
substrate rhodamine-123 following its intracameral injection in rabbits is ~4-fold greater than clearance due to passive aqueous humor outflow. Additionally, they found a 50% reduction in the aqueous humor clearance of rhodamine-123 following co-administration of the Pgp inhibitor quinidine (Kajikawa, et al., 2000). These results are similar to those of Senthilkumari et al. (Senthilkumari, et al., 2008), who showed following intravitreal injection a 2.6-fold increase in vitreous rhodamine-123 levels with co-administration of the Pgp inhibitor, elacridar. Probably the most definitive evidence for a role for Pgp in blood-aqueous humor barrier function in ocular drug disposition comes from a study done by Fujii et al (Fujii, et al., 2014) using Mdr1a knockout (Pgp deficient) rats. Following intravenous administration in vivo of quinidine, digoxin and verapamil, the aqueous humor uptake index values and the in vivo blood-to-tissue influx permeability clearances of these Pgp substrates across the blood-aqueous humor barrier were higher in Mdr1a knockout rats compared to wild-type rats (Fujii, et al., 2014).

In addition to Pgp substrates, several organic anions are actively eliminated from the eye in vivo. The elimination of carbenecillin (Barza, et al., 1982), fluorescein and fluorescein monoglucononide (Kitano and Nagataki, 1986), and iodopyracet (Forbes and Becker, 1960) from the rabbit eye, and cefazolin and carbenecillin from the monkey eye (Barza, et al., 1983) following intravitreal administration are reduced following concomitant administration of probenecid, a prototypical inhibitor of many drug transporters that interact with organic anions (e.g., OATs, OATPs and MRPs). Furthermore, the elimination of iodopyracet from the rabbit eye is a saturable process with a secretory maximum, consistent with the presence of active transport (Becker and Forbes, 1961).

Although these in vivo data do not directly implicate a contribution of the iris-ciliary body in limiting drug exposure in the eye, the expression of a variety of drug transporters in this
tissue, and the presence of their activity in in vitro preparations, strongly suggest that the tissue performs this function. It is important to note that the blood-retinal barrier and cornea also express many drug transporters, and are most certainly important for ocular drug disposition.

The question remains, which blood ocular barrier is most important to ocular drug disposition? The barrier most important is likely dependent on a variety of factors, including the site of drug administration (topical, systemic, intravitreal or intracameral), the surface area of the tissues, barriers to drug diffusion (e.g., unstirred fluid layers), and the abundance and activity of drug transporters in these tissues.

Perspectives

The data presented indicate that the iris-ciliary body expresses a variety of drug transporters that are likely involved in limiting drug exposure in the eye. However, it may be possible to ‘high jack’ select drug transporters in order to enhance ocular drug bioavailability. Indeed, drug transporters in other tissues have been targeted to improve the pharmacokinetic profile of drugs. For example, PEPT1 has been targeted to increase the oral bioavailability of the acyclovir pro-drug, valacyclovir (MacDougall and Guglielmo, 2004). Could PEPT1 in the iris-ciliary body be used to increase the ocular bioavailability of acyclovir for treatment of ocular herpes virus infections? To take advantage of drug transporters in order to enhance ocular drug bioavailability will require a better understanding of their cellular and subcellular distribution in the iris-ciliary body, and the predominant direction in which they transport their drug substrates.

Although the focus of this review is on drug transporters and their influence on ocular pharmacokinetics, many of the transporters present in the iris-ciliary body play important physiological roles, yet we lack an understanding of their importance in ocular physiology. For example, what purpose do ergothioneine (OCTN1), carnitine (OCTN2), peptide (PEPT1 and
PEPT2) and cyclic nucleotide (MRP4 and MRP5) transporters serve in the iris-ciliary body? Ergothioneine is a naturally occurring amino acid derivative that has antioxidant properties. Although it is not made in the human body, some human tissues contain high concentrations of ergothioneine. Relatively high levels of ergothioneine are present in bovine and porcine ocular tissues, including aqueous humor, where it likely protects ocular tissues from oxidative damage (Shires, et al., 1997). Carnitine is important for conversion of fatty acids into metabolic energy, and as an antioxidant. Within the eye of animals levels of ω-carnitine are highest in the iris, ciliary body, and choroid-retina, and the use of ω-carnitine and its derivatives in humans may potentially slow age-related ocular pathologies, such as age-related macular degeneration (Pescosolido, et al., 2008). Future work should examine the influence of OCTN1 and OCTN2 on levels of ergothioneine and carnitine in ocular tissues. Aqueous humor contains a variety of components that absorb ultraviolet radiation (UV), including ascorbate, uric acid, proteins and the amino acids tyrosine and tryptophan (Ringvold, et al., 2003). Perhaps peptide transporters in the iris-ciliary body contribute to the UV-absorbing potential of aqueous humor. The cAMP-adenosine signaling pathway modulates aqueous humor inflow (Farahbakhsh, 2003). The ability of MRP4 and MRP5 to modulate intracellular and extracellular cAMP levels may make these transporters novel targets for glaucoma therapy. A better understanding of the role drug transporters in the blood-aqueous humor barrier play in ocular pharmacokinetics and physiology should lead to improved treatment of ocular diseases.
Authorship Contributions

Wrote or contributed to the writing of the manuscript: JL and RMP
References


Figure Legends

Figure 1. Schematic diagram of the anterior globe highlighting ABC and SLC drug transporters shown to be expressed in dissections of human iris-ciliary body, ciliary body or iris by immunoblotting. See text for references to the published studies.

Figure 2. Cellular and subcellular expression of protein for ABC and SLC drug transporters in the human ciliary body (left panel) and iris (right panel) determined by immunohistochemistry. *, indicates discrepancies between studies (see text). BLM, basolateral membrane; NPE, non-pigmented epithelium; PC, pigmented cell. See text for references to the published studies.
Table 1. Expression of mRNA for ABC drug transporters in iris-ciliary body (ICB), ciliary body (CB) or iris (I) from human.

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Chen ICB</th>
<th>Zhang ICB</th>
<th>Dahlin CB</th>
<th>Lee CB</th>
<th>Dahlin I</th>
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The studies by Chen et al. (Chen et al., 2013), Dahlin et al. (Dahlin et al., 2013), Lee et al. (Lee et al., 2015), Pelis et al. (Pelis et al., 2009) and Zhang et al. (Zhang et al., 2008) used qPCR, microarray, microarray, RT-PCR and qPCR for mRNA detection, respectively. -, not detected. +, detected. +/-, indicates that mRNA expression was low to negligible. Blank areas indicate that the study did not examine expression of the respective ABC drug transporter. The number of individual donor eyes used in these studies are as follows: Chen et al. (n=6), Dahlin et al. (n=15), Lee et al. (n=5-6), Pelis et al. (n=1) and Zhang et al. (n=3).
Table 2. Expression of mRNA for SLC drug transporters in iris-ciliary body (ICB), ciliary body (CB) or iris (I) from human.

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The studies by Zhang et al. (Zhang et al., 2008), Dahlin et al. (Dahlin et al., 2013) and Lee et al. (Lee et al., 2015) used qPCR, microarray and microarray for mRNA detection, respectively. -, not detected. +, detected. +/-, indicates that mRNA expression was low to negligible. Blank areas indicate that the study did not examine expression of the respective SLC drug transporter.
The number of individual donor eyes used in these studies are as follows: Dahlin et al. (n=15), Lee et al. (n=5-6) and Zhang et al. (n=3).
Figure 1

- MRP1, MRP2, MRP4, Pgp, BCRP, OATP1A2, OATP1C1, OATP2B1, OATP3A1, OATP4A1, OAT1, OAT3, NaDC3

- Iris-ciliary body
  - MRP1, MRP2, MRP4, BCRP

- Iris:
  - MRP1, MRP2, MRP3, MRP4, MRP6, MRP7, Pgp

- Ciliary body
  - MRP1, MRP2, MRP4, BCRP

- Aqueous humor
- Lens
- Retinal pigmented epithelium
- Vitreous humor

- Tight endothelial cells
- Fenestrated endothelial cells
- Retinal pigmented epithelium
- Ciliary epithelium (bilayer)