

Transporter-targeted Drug Delivery to the Retina

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The unique anatomy and physiology of the eye make ocular drug delivery a challenge for pharmaceutical scientists. Various ocular barriers prevent agents from achieving therapeutic concentration levels at the target tissues. More important, drug delivery to the back of the eye poses even stiffer challenges, including the site of origin and progression of various sight-threatening diseases such as diabetic macular edema, diabetic retinopathy, wet and dry age-related macular degeneration, cytomegalovirus retinitis and proliferative vitreoretinopathy. Conventional delivery routes, such as topical and systemic modes of administration may not deliver effective doses of the drug to the target tissues in the posterior segment.^{1,2}

TOPICAL AND SYSTEMIC THERAPY

Upon instillation of topical eye drops, precorneal factors such as solution drainage, blinking, tear turnover rate, and nonproductive absorption by adjacent tissues (conjunctiva and lacrimal gland), drastically reduce precorneal drug concentration. In addition, efflux pumps such as P-glycoprotein (P-gp) and multidrug resistance-associated proteins (MRP) combined with the inherent tight junctions on the corneal epithelium significantly hinder drug absorption across the cornea.³⁻⁸ Further impedance to reaching the posterior segment is caused by the lens and by aqueous humor outflow, the direction of which is against the concentration gradient. Hence, topical drug administration is often inadequate for retinal drug delivery, despite high patient acceptance and compliance.

Therapeutic agents, when administered systemically, also demonstrate limited absorption into the retina and vitreous. The choroid is richly perfused with blood ves-

sels, but blood-ocular barriers such as the blood-aqueous barrier (BAB) and blood-retinal barrier (BRB), limit the entry of molecules from the systemic circulation into ocular chambers and tissues.⁹ The BRB is primarily composed of retinal endothelial blood vessels and retinal pigment epithelium (RPE), which constitute a significant barrier to drug absorption into the retina and vitreous.¹⁰ Permeabilities of molecules across the BRB are comparable with those across the blood-brain barrier (BBB), which underscores its effectiveness in restricting the entry of small molecules into the retina.¹¹ Moreover, the RPE, otherwise known as the outer BRB, expresses efflux pumps such as P-gp and MRP, which further limit the permeation of various xenobiotics and endogenous compounds from the choroid into the retina and vitreous.^{12,13} P-gp is also reported to be expressed on the retinal endothelial vessels, otherwise known as the inner BRB.¹⁴

INTRAVITREAL AND PERIOCCULAR INJECTIONS

Other delivery routes to the posterior segment include intravitreal and periocular injection. Intravitreal mode of administration is sought in critical disease conditions, that require direct injection into the vitreous humor through the pars plana, evading all the barriers. Even though this route can achieve higher drug concentrations in the neural retina, adverse effects such as retinal detachment from repeated injections, retinal hemorrhage, endophthalmitis, and other retinal toxicities due to high concentrations upon bolus dose administration may result in poor patient acceptance and compliance.^{1,15,16} Periocular injection refers to placing the drug in the region surrounding the eye. Of all the existing routes, periocular drug administration is considered to be relatively efficient, though not completely noninvasive.

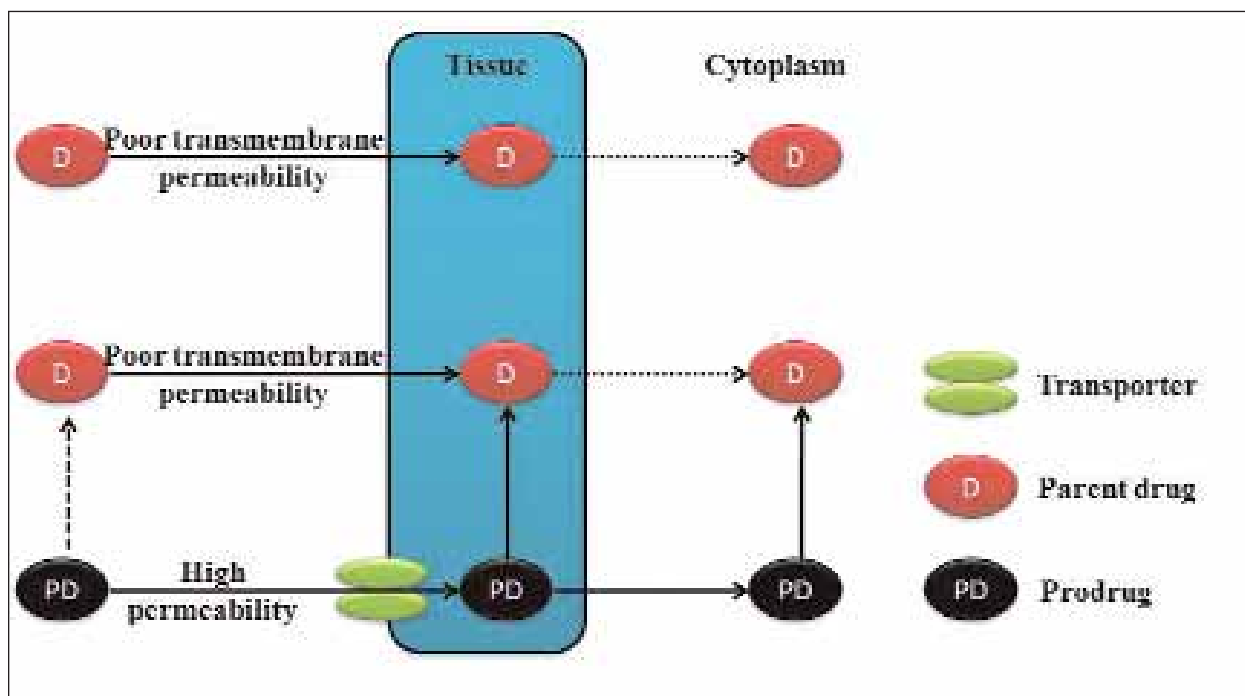


Figure 1. Schematic representation of prodrug approach.

The current challenge facing drug delivery scientists and ocular pharmacologists is to deliver therapeutic agents to target tissues by effectively circumventing the unique anatomical barriers of the eye, without causing any patient discomfort or alteration in protective physiologic mechanisms. In this regard, targeting nutrient transporters on ocular barriers utilizing a prodrug approach offers much promise.

TRANSPORTER TARGETED PRODRUG APPROACH: THE CONCEPT

Nutrient transporters are transmembrane proteins involved in the translocation of essential nutrients and xenobiotics across biological membranes, thereby regulating the supply of essential ingredients into cells. Several transporters and receptors for nutrients and endogenous compounds are expressed on both the apical and basolateral sides of the epithelial barrier of various tissues such as intestine, kidney, BBB, BRB, and placenta. To take advantage of the nutrient transport systems, the parent drug may be covalently conjugated to the nutrient moiety by an enzymatically cleavable bond generating a prodrug. Prodrugs or analogs designed to target these transporters can significantly enhance absorption of poorly permeating parent drug. Solubility, desired membrane permeability and evasion of efflux pumps can be simultaneously achieved by proper selection of a promoiety. These prodrugs are recognized by the membrane trans-

porters as substrates and are translocated across the epithelial or endothelial barrier. Subsequently, the prodrugs are enzymatically cleaved to release the parent drug and the ligand, which in most cases is a nutrient, nontoxic and easily eliminated. A schematic representation of transporter-mediated drug delivery across a cell barrier is illustrated in Figure 1.

TARGETING TRANSPORTERS ON THE RETINA

Carriers for peptides, amino acids, monocarboxylic acids, folates, nucleosides and nucleobases, organic anions, and cations are a few examples of nutrient transporters that can usually be targeted for drug delivery.¹⁷ Current literature reports describe a host of such nutrient carriers being expressed on the retina—more precisely, on the RPE and retinal endothelial cells, which in turn can be utilized to target and deliver drugs to the vitreoretinal spaces.^{18–25} These transporters can be targeted following systemic, intravitreal and periocular routes of administration. Transporters expressed on the RPE can play a significant role in the translocation of the prodrug and thereby elevate the concentration of the prodrug/drug in the retina following systemic and periocular administration. A schematic representation of this approach is shown in Figures 2A and 2B. Prodrugs administered intravitreally could be recognized by the nutrient transporters expressed on the retinal endothe-

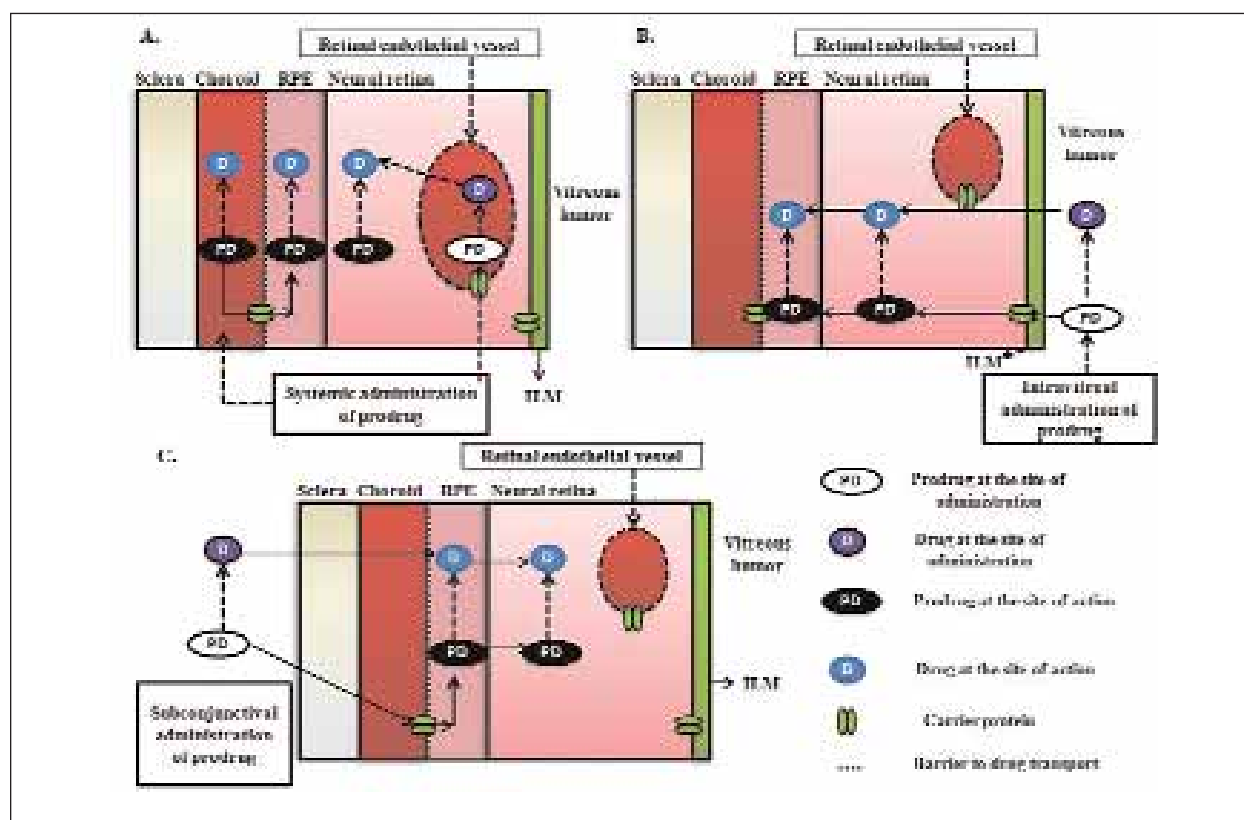


Figure 2. Schematic delineating transporter prodrug approach via systemic administration: (A) intravitreal administration (B) and periocular administration (C).

lial cells and thereby translocate prodrugs across neural retina across the RPE (Figure 2C). Subsequently, prodrugs are enzymatically cleaved at the target site to release the parent drug, which elicits the desired therapeutic response. Although a number of nutrient transporters on the retina have been identified and characterized, the current article discusses the peptide and amino acid transporters, which demonstrate the greatest promise with respect to drug delivery because of their wide substrate acceptability and high capacity.

PEPTIDE TRANSPORTERS

Peptide transporters are responsible for the translocation of dipeptides, tripeptides, and peptidomimetic drugs across various epithelia.²⁶ These transporters are proton-coupled and are mainly classified into PepT1, PepT2, and peptide/histidine transporters (PHT1 and PHT2), differing slightly in their tissue distribution, localization, affinity, and capacity to transport substrates. These transporters have gained attention for targeted drug delivery in recent years because of their relatively high capacity to ferry molecules across lipoidal membranes combined with a lower structural

specificity requirement compared with other known transporters. Many structurally diverse drugs with varied pharmacologic activities can be delivered to cells utilizing these transporters. Examples of PepT substrates include cephalosporins, angiotensin-converting enzyme (ACE) inhibitors, β -lactam antibiotics, rennin inhibitors, and a few other compounds without a peptide bond such as 5-aminolevulinic acid.^{27,28} High oral bioavailability of therapeutic agents such as acyclovir, ganciclovir, and zidovudine is attributed to prodrug derivatization (ie, esterification with di- or tripeptides).²⁹ In addition, valine ester prodrugs of acyclovir and ganciclovir, namely, valacyclovir and valganciclovir, also are translocated by the intestinal peptide transporter PepT1. As a result of valine conjugation, oral bioavailability of acyclovir increased by three- to five-fold and ganciclovir by almost tenfold.^{30,31} Such derivatization has also led to a drastic increase in the aqueous solubility of acyclovir and ganciclovir, allowing more flexibility in formulation design.

These peptide transporters have also been found to be expressed in various ocular tissues, such as cornea, retina and BAB.^{21,32,33} Corneal permeation of acyclovir

and ganciclovir was significantly elevated due to dipeptide conjugation to the parent drug, resulting in enhanced therapeutic activity against herpes viruses.^{32,34} Presence of a peptide transporter on BAB was reported from our laboratory. Significant transport of a model (glycylsarcosine) PepT substrate, following systemic administration provided the initial observation that PepT might be expressed on BAB.³³ A time- and concentration- dependent, carrier-mediated uptake of glycylsarcosine across the blood-ocular barrier was reported. Prodrugs of acyclovir exhibited higher concentrations in the aqueous humor following systemic administration relative to the parent drug.³⁵ More recent work in our laboratory reported significantly higher levels of acyclovir following systemic administration of stereoisomeric dipeptide prodrug of acyclovir (L-val-D-val-acyclovir) on the cornea, relative to the parent moiety, providing additional evidence for the presence of PepT on BAB.³⁶ In the retina, the presence of PepT has been reported on RPE and retinal endothelial cells. The transporter in the neural retina, facing the vitreous humor, could be targeted following intravitreal administration, whereas the one on the RPE could be targeted for drug delivery following systemic and periocular routes. Recent studies have shown the expression of PHT1 on human and bovine RPE cells as well as neural retina.³⁷ Expression of PepT1 was not detected, although PepT2 and PHT2 are known to be expressed on bovine and human retina.³⁷ Recent work from our laboratory has demonstrated that the oligopeptide transport system on the RPE leads to at least twofold increase in the permeation of PepT targeted prodrugs of ganciclovir (valine-GCV, valine-valine-GCV and glycine-valine-GCV) across retina-choroid-sclera (RCS, S → R direction) relative to the parent drug, ganciclovir.²¹ Following translocation by PepT, these prodrugs are subsequently cleaved by enzymes, particularly the cholinesterases, dipeptidases, and aminopeptidases, present in the retina and vitreous.

To summarize, peptide transporters are extremely versatile and important targets for drug delivery to ocular tissues.

AMINO ACID TRANSPORTERS

Amino acids are required in the protein synthesis of all living cells. Due to their hydrophilicity, membrane proteins exist to transport essential amino acids across lipoidal membranes. Amino acid transporters constitute a large family of membrane transporters classified based on their substrate specificity, charge, and dependence on sodium ion cotransport.^{38,39} These proteins are broadly

classified as cationic, anionic, and neutral amino acid transporters and are further subclassified into sodium dependent and independent transporters. System L, a large amino acid transporter, system y⁺, a cationic amino acid transporter, and system b^{0,+}, a cationic and neutral amino acid transporter, belong to sodium-independent transporters while system X⁻, an anionic transporter; system A, B⁰, B^{0,+}, ASC (alanine-serine-cystine) and β-amino acid transporters belong to the sodium-dependent category. A large neutral amino-acid transport (LAT) system exists in two isoforms, LAT1 and LAT2, which differ in their substrate specificity. LAT1 is mainly involved in the transport of large neutral amino acids, such as Leu, Phe, Ile, Trp, Val, Tyr, His, and Met, and LAT2 transports both large neutral amino acids and small neutral amino acids.

Drugs such as gabapentin, L-dopa, and α-methyl-dopa are some of the substrates of amino-acid transporters, which are expressed in many tissues, including the eye.⁴⁰ Presence of sodium-independent systems, such as LAT1, and sodium-dependent systems, such as B^{0,+} and ASCT1 transporters, have been reported in rabbit corneal epithelial cells.⁴⁰⁻⁴³ Our laboratory reported the synthesis of a series of prodrugs of acyclovir (ACV) targeting the amino acid transporters, such as alanine-ACV, serine-ACV, isoleucine-ACV, γ-glutamyl-ACV, and valine-ACV, with an aim to improve the corneal permeability and in turn ocular bioavailability of acyclovir.⁴⁴ Serine-ACV exhibited a better pharmacokinetic profile relative to the parent drug resulting in a significantly higher AUC, C_{max}, and C_{last} values, owing to transporter mediated translocation across corneal epithelium.⁴⁴ In the posterior segment, molecular evidence and functional activity of LAT2 (sodium-independent) has been shown in ARPE-19 cells (human retinal pigment epithelial cells).¹⁹

Other carriers for amino acids in the RPE as well as retinal endothelial cells are currently under investigation. Eventually, these transporters could be utilized for targeting prodrugs to enhance absorption of poorly permeable drugs across ocular barriers following intravitreal, systemic, and periocular routes of administration.

EVASION OF EFFLUX PUMPS BY PRODRUG DERIVATIZATION

Multidrug resistance efflux pumps belonging to the ATP-binding cassette (ABC) superfamily of membrane transporters such as P-gp, MRP, and breast cancer resistance protein (BCRP) can restrict the bioavailability of many drugs in various tissues by pumping agents from the lipid bilayer or cytoplasm back into the extracellular fluid. Recently, such efflux pumps have also been report-

ed to be expressed in a variety of ocular tissues. Cells of the retinal capillary endothelium, RPE, conjunctival epithelium, iris-ciliary muscle, and corneal epithelium are known to express P-gp.^{4,45-48} Presence of MRP has also been reported on the blood side of bovine RPE by Schlosshauer and colleagues.¹¹ Cornea has also been shown to express a host of MRP isoforms, such as MRP1, MRP2, MRP3, and MRP5.⁶⁻⁸ Expression of efflux pumps on the cornea and retinal pigment epithelium could be correlated with a defensive mechanism for the entry and accumulation of xenobiotics from the external environment and the systemic circulation, respectively.

From a drug delivery point of view, these efflux transporters lower absorption of substrate molecules across various epithelia, thereby restricting their bioavailability. P-gp expressed on the RPE was shown to be functionally active by effluxing out Rhodamine 123, a P-gp substrate, from the neural retina into the choroidal blood supply.¹¹ Efflux of another P-gp substrate, quinidine, was shown to be modulated in the presence of specific efflux modulators following systemic and intravitreal administration, which suggests the presence of a functionally active P-gp on the RPE and the neural retina.⁴⁹

Therefore, these efflux pumps must be effectively circumvented to enhance drug absorption across the retina. Prodrug derivatization of a drug substrate targeting an influx nutrient transporter offers great potential. In this strategy, prodrugs are designed such that the modified compounds become substrates of nutrient transporters, leading to enhanced absorption across epithelial barriers. In addition, efflux is effectively circumvented due to diminished or no affinity of the drug molecule towards efflux pumps due to structural modification and binding to the influx transporter. We reported that peptide prodrug derivatization of quinidine, a P-gp substrate, demonstrates diminished affinity towards efflux pump. Effective circumvention of P-gp on the corneal epithelium thereby led to higher corneal permeability of quinidine prodrugs relative to the parent drug.⁵⁰ Similar results have also been observed with peptide and amino acid prodrugs of the HIV protease inhibitors saquinavir and lopinavir, which suffer from poor oral bioavailability being a substrate of multidrug resistance efflux pumps as well as drug metabolizing enzymes (CYP3A4).⁵¹⁻⁵³ Prodrug derivatization of these molecules diminished their affinity toward efflux pumps and CYP3A4, resulting in higher oral bioavailability.

Thus, rational prodrug design can not only enhance passive diffusion, but can also lead to decreased recognition by efflux pumps, leading to reduced multidrug resistance mediated efflux and higher absorption and bioavailability.

CONCLUSION AND FUTURE OUTLOOK

Significant advances in the field of transporter-targeted delivery have been achieved in the past decade.

Valacyclovir and valganciclovir are examples of two well-known marketed drugs utilizing this approach. A number of transporters have been identified on the retina, both on the RPE and retinal endothelial cells, which could be exploited to enhance retinal bioavailability. Among them, peptide and amino acid transporters hold the greatest potential due to wide substrate specificity and high capacity in translocating substrates. In general, prodrugs may be designed by conjugating appropriate promoieties with an aim to improve lipophilicity (↑ absorption through lipid bilayers) and water solubility (↑ formulation feasibility). Moreover, the ability of prodrugs to bypass multidrug resistance efflux may further enhance bioavailability via prodrug derivatization.

The periocular route presents an attractive alternative to conventional routes of drug delivery to the retina, ie, intravitreal, topical and systemic administration. This route is partially invasive and results in ocular complications upon repeated injections. But such complications could be significantly overcome by designing novel sustained-release formulations, thereby drastically reducing the frequency of periocular injections. Drug delivery utilizing nanoparticles prepared from novel polymers suspended in a thermosensitive gel will be a valuable strategy for development of a controlled-release formulation. Thermosensitive gel of polymers can prolong the sustained release of drugs at the target site. Nanoparticles suspended in a thermosensitive gel could minimize the burst release of drugs that is a common problem encountered with conventional nanoparticles. Moreover, if prodrugs targeting nutrient transporters on the retina are encapsulated in such novel sustained release delivery systems, then a dual advantage is realized: ie, enhanced cellular uptake through evasion of multidrug resistance efflux and prolonged duration of action. Future work in retinal drug delivery will focus on more noninvasive drug delivery systems through periocular routes that can provide drug levels for long periods of time after a single administration. ■

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1. Duvvuri S, Majumdar S, Mitra AK. Drug delivery to the retina: challenges and opportunities. *Expert Opin Biol Ther.* 2003;3:45–56.
2. Hughes PM, Olejnik O, Chang-Lin JE, Wilson CG. Topical and systemic drug delivery to the posterior segments. *Adv Drug Deliv Rev.* 2005; 57:2010–2032.
3. Dey S, Gunda S, Mitra AK. Pharmacokinetics of erythromycin in rabbit corneas after single-dose infusion: role of P-glycoprotein as a barrier to in vivo ocular drug absorption. *J Pharmacol Exp Ther.* 2004;311:246–255.
4. Dey S, Patel J, Anand BS, Jain-Vakkalagadda B, et al. Molecular evidence and functional expression of P-glycoprotein (MDR1) in human and rabbit cornea and corneal epithelial cell lines. *Invest Ophthalmol Vis Sci.* 2003;44:2909–2918.
5. Grass GM, Robinson JR. Mechanisms of corneal drug penetration. II: Ultrastructural analysis of potential pathways for drug movement. *J Pharm Sci.* 1988;77:15–23.
6. Hariharan S, Gunda S, Mishra GP, Pal D, Mitra AK. Enhanced corneal absorption of erythromycin by modulating P-glycoprotein and MRP mediated efflux with corticosteroids. *Pharm Res.* 2009;26:1270–1282.
7. Karla PK, Pal D, Mitra AK. Molecular evidence and functional expression of multidrug resistance associated protein (MRP) in rabbit corneal epithelial cells. *Exp Eye Res.* 2007;84:53–60.
8. Karla PK, Quinn TL, Herndon BL, Thomas P, Pal D, Mitra A. Expression of multidrug resistance-associated protein 5 (MRP5) on cornea and its role in drug efflux. *J Ocul Pharmacol Ther.* 2009;25:121–132.
9. Cunha-Vaz J. The blood-ocular barriers. *Surv Ophthalmol.* 1979;23:279–296.
10. Cunha-Vaz JG. The blood-retinal barriers. *Doc Ophthalmol.* 1976;41:287–327.
11. Steuer H, Jaworski A, Elger B, et al. Functional characterization and comparison of the outer blood-retina barrier and the blood-brain barrier. *Invest Ophthalmol Vis Sci.* 2005;46:1047–1053.
12. Aukunuru JV, Sunkara G, Bandi N, Thoreson WB, Kompella UB. Expression of multidrug resistance-associated protein (MRP) in human retinal pigment epithelial cells and its interaction with BAPSG, a novel aldose reductase inhibitor. *Pharm Res.* 2001;18:565–572.
13. Kennedy BG, Mangini NJ. P-glycoprotein expression in human retinal pigment epithelium. *Mol Vis.* 2002;8:422–430.
14. Greenwood J. Characterization of a rat retinal endothelial cell culture and the expression of P-glycoprotein in brain and retinal endothelium in vitro. *J Neuroimmunol.* 1992;39:123–132.
15. Baum J, Peyman GA, Barza M. Intravitreal administration of antibiotic in the treatment of bacterial endophthalmitis. III. Consensus. *Surv Ophthalmol.* 1982;26:204–206.
16. Cantrill HL, Henry K, Melroe NH, Knobloch WH, Ramsay RC, Balfour HH, Jr. Treatment of cytomegalovirus retinitis with intravitreal ganciclovir. Long-term results. *Ophthalmology.* 1989;96:367–374.
17. Lee VH. Membrane transporters. *Eur J Pharm Sci.* 2000;11(Suppl 2):S41–S50.
18. Dun Y, Mysona B, Itagaki S, Martin-Studdard A, Ganapathy V, Smith SB. Functional and molecular analysis of D-serine transport in retinal Muller cells. *Exp Eye Res.* 2007;84:191–199.
19. Gandhi MD, Pal D, Mitra AK. Identification and functional characterization of a Na⁺/independent large neutral amino acid transporter (LAT2) on ARPE-19 cells. *Int J Pharm.* 2004;275:189–200.
20. Gerhart DZ, Leino RL, Drewes LR. Distribution of monocarboxylate transporters MCT1 and MCT2 in rat retina. *Neuroscience.* 1999;92:367–375.
21. Kansara V, Hao Y, Mitra AK. Dipeptide monoester ganciclovir prodrugs for transscleral drug delivery: targeting the oligopeptide transporter on rabbit retina. *J Ocul Pharmacol Ther.* 2007;23:321–334.
22. Kansara V, Luo S, Balasubrahmanyam B, Pal D, Mitra AK. Biotin uptake and cellular translocation in human derived retinoblastoma cell line (Y-79): a role of hSMVT system. *Int J Pharm.* 2006;312:43–52.
23. Kansara V, Pal D, Jain R, Mitra AK. Identification and functional characterization of riboflavin transporter in human-derived retinoblastoma cell line (Y-79): mechanisms of cellular uptake and translocation. *J Ocul Pharmacol Ther.* 2005;21:275–287.
24. Kansara V, Paturi D, Luo S, Gaudana R, Mitra AK. Folic acid transport via high affinity carrier-mediated system in human retinoblastoma cells. *Int J Pharm.* 2008;355:210–219.
25. Wiessner M, Fletcher EL, Fischer F, Rauert T. Localization and possible function of the glutamate transporter, EAAC1, in the rat retina. *Cell Tissue Res.* 2002;310:31–40.
26. Adibi SA. Renal assimilation of oligopeptides: physiological mechanisms and metabolic importance. *Am J Physiol.* 1997;272:E723–736.
27. Novotny A, Xiang J, Stummer W, Teuscher NS, Smith DE, Keep RF. Mechanisms of 5-aminolevulinic acid uptake at the choroid plexus. *J Neurochem.* 2000;75:321–328.
28. Sala-Rabanal M, Loo DD, Hirayama BA, Turk E, Wright EM. Molecular interactions between dipeptides, drugs and the human intestinal H⁺-oligopeptide cotransporter hPEPT1. *J Physiol.* 2006;574:149–166.
29. Anand BS, Patel J, Mitra AK. Interactions of the dipeptide ester prodrugs of acyclovir with the intestinal oligopeptide transporter: competitive inhibition of glycylsarcosine transport in human intestinal cell line-Caco-2. *J Pharmacol Exp Ther.* 2003;304:781–791.
30. Brown F, Banken L, Saywell K, Arum I. Pharmacokinetics of valganciclovir and ganciclovir following multiple oral dosages of valganciclovir in HIV- and CMV-seropositive volunteers. *Clin Pharmacokinet.* 1999;37:167–176.
31. Soul-Lawton J, Seaber E, On N, Wootton R, Rolan P, Posner J. Absolute bioavailability and metabolic disposition of valganciclovir, the L-valyl ester of acyclovir, following oral administration to humans. *Antimicrob Agents Chemother.* 1995;39:2759–2764.
32. Anand BS, Mitra AK. Mechanism of corneal permeation of L-valyl ester of acyclovir: targeting the oligopeptide transporter on the rabbit cornea. *Pharm Res.* 2002;19:1194–1202.
33. Atluri H, Anand BS, Patel J, Mitra AK. Mechanism of a model dipeptide transport across blood-ocular barriers following systemic administration. *Exp Eye Res.* 2004;78:815–822.
34. Gunda S, Hariharan S, Mitra AK. Corneal absorption and anterior chamber pharmacokinetics of dipeptide monoester prodrugs of ganciclovir (GCV): in vivo comparative evaluation of these prodrugs with Val-GCV and GCV in rabbits. *J Ocul Pharmacol Ther.* 2006;22:465–476.
35. Dias C, Nashed Y, Atluri H, Mitra A. Ocular penetration of acyclovir and its peptide prodrugs valganciclovir and val-valacyclovir following systemic administration in rabbits: An evaluation using ocular microdialysis and LC-MS. *Curr Eye Res.* 2002;25:243–252.
36. Talluri RS, Gaudana R, Hariharan S, Mitra AK. Pharmacokinetics of stereoisomeric dipeptide prodrugs of acyclovir following intravenous and oral administrations in rats: A study involving corneal uptake following oral administration. Manuscript in preparation.
37. Ocheltree SM, Keep RF, Shen H, Yang D, Hughes BA, Smith DE. Preliminary investigation into the expression of proton-coupled oligopeptide transporters in neural retina and retinal pigment epithelium (RPE): lack of functional activity in RPE plasma membranes. *Pharm Res.* 2003;20:1364–1372.
38. Christensen HN. Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev.* 1990;70:43–77.
39. Tamai I, Tsuji A. Transporter-mediated permeation of drugs across the blood-brain barrier. *J Pharm Sci.* 2000;89:1371–1388.
40. Wheeler G. Gabapentin. Pfizer. *Curr Opin Investig Drugs.* 2002;3:470–477.
41. Jain-Vakkalagadda B, Dey S, Pal D, Mitra AK. Identification and functional characterization of a Na⁺-independent large neutral amino acid transporter, LAT1, in human and rabbit cornea. *Invest Ophthalmol Vis Sci.* 2003;44:2919–2927.
42. Jain-Vakkalagadda B, Pal D, Gunda S, Nashed Y, Ganapathy V, Mitra AK. Identification of a Na⁺-dependent cationic and neutral amino acid transporter, B(0,+), in human and rabbit cornea. *Mol Pharm.* 2004;1:338–346.
43. Katragadda S, Talluri RS, Pal D, Mitra AK. Identification and characterization of a Na⁺-dependent neutral amino acid transporter, ASCT1, in rabbit corneal epithelial cell culture and rabbit cornea. *Curr Eye Res.* 2005;30:989–1002.
44. Katragadda S, Gunda S, Hariharan S, Mitra AK. Ocular pharmacokinetics of acyclovir amino acid ester prodrugs in the anterior chamber: evaluation of their utility in treating ocular HSV infections. *Int J Pharm.* 2008;359:15–24.
45. Holash JA, Stewart PA. The relationship of astrocyte-like cells to the vessels that contribute to the blood-ocular barriers. *Brain Res.* 1993;629:218–224.
46. Kawazu K, Yamada K, Nakamura M, Ota A. Characterization of cyclosporin A transport in cultured rabbit corneal epithelial cells: P-glycoprotein transport activity and binding to cyclophilin. *Invest Ophthalmol Vis Sci.* 1999;40:1738–1744.
47. Saha P, Yang JJ, Lee VH. Existence of a p-glycoprotein drug efflux pump in cultured rabbit conjunctival epithelial cells. *Invest Ophthalmol Vis Sci.* 1998;39:1221–1226.
48. Schlingemann RO, Hofman P, Klooster J, Blaauwgeers HG, Van der Gaag R, Vrensen GF. Ciliary muscle capillaries have blood-tissue barrier characteristics. *Exp Eye Res.* 1998;66:747–754.
49. Duvvuri S, Gandhi MD, Mitra AK. Effect of P-glycoprotein on the ocular disposition of a model substrate, quinidine. *Curr Eye Res.* 2003;27:345–353.
50. Katragadda S, Talluri RS, Mitra AK. Modulation of P-glycoprotein-mediated efflux by pro-drug derivatization: an approach involving peptide transporter-mediated influx across rabbit cornea. *J Ocul Pharmacol Ther.* 2006;22:110–120.
51. Agarwal S, Boddu SH, Jain R, Samanta S, Pal D, Mitra AK. Peptide prodrugs: improved oral absorption of lopinavir, a HIV protease inhibitor. *Int J Pharm.* 2008;359:7–14.
52. Jain R, Agarwal S, Majumdar S, Zhu X, Pal D, Mitra AK. Evasion of P-gp mediated cellular efflux and permeability enhancement of HIV-protease inhibitor saquinavir by prodrug modification. *Int J Pharm.* 2005;303:8–19.
53. Jain R, Duvvuri S, Kansara V, Mandava NK, Mitra AK. Intestinal absorption of novel-dipeptide prodrugs of saquinavir in rats. *Int J Pharm.* 2007;336:233–240.