Expert Opinion

Strategies for therapy of retinal diseases using systemic drug delivery: relevance of transporters at the blood–retinal barrier

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Introduction: There is an increasing need for managing rapidly progressing retinal diseases because of the potential loss of vision. Although systemic drug administration is one possible route for treating retinal diseases, retinal transfer of therapeutic drugs from the circulating blood is strictly regulated by the blood–retinal barrier (BRB).

Areas covered: This review discusses the constraints and challenges of drug delivery to the retina. In addition, this article discusses the properties of drugs and the conditions of the BRB that affect drug permeability. The reader will gain insights into the strategies for developing therapeutic drugs that are able to cross the BRB for treating retinal diseases. Further, the reader will gain insights into the role of BRB physiology including barrier functions, and the effect of influx and efflux transporters on retinal drug delivery.

Expert opinion: When designing and selecting optimal drug candidates, it's important to consider the fact that they should be recognized by influx transporters and that efflux transporters at the BRB should be avoided. Although lipophilic cationic drugs are known to be transported to the brain across the blood–brain barrier, verapamil transport to the retina is substantially higher than to the brain. Therefore, lipophilic cationic drugs do have a great ability to increase influx transport across the BRB.

Keywords: ABC transporters, drug delivery, inner blood–retinal barrier, outer blood–retinal barrier, retina, SLC transporters

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1. Introduction

The most prevalent retinal diseases which cause visual impairment include glaucoma, diabetic retinopathy, cytomegalovirus retinitis, endophthalmitis and age-related macular degeneration [1-3]. In recent times, there has been an increased understanding of the disease processes that affect the retina and improved diagnostic techniques and the discovery of new antiangiogenic compounds and neuroprotectants [1,4]. Delivery of therapeutic drugs to the retina is faced with several hurdles. Topical application of drugs (eye drops) is ineffective in producing therapeutic concentrations in the retina because of the longer diffusional distance and counterdirectional intraocular convection from the ciliary body to Schlemm's canal [5]. Invasive methods, such as intravitreal and intrascleral delivery, take the drug close to the retina (**Figure 1**). However, intravitreal delivery with implants and direct injections carries a high risk of deleterious side effects, such as postoperative endophthalmitis, hemorrhage and retinal detachment [6,7]. Intrascleral delivery with direct injections also has disadvantages like the need for repeated administrations, side effects including mild pain, and prevention of retinal transfer of drugs from the sclera by retinal pigment epithelial

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Article highlights.

- The blood-retinal barrier (BRB) is composed of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (outer BRB).
- The inner and outer BRB are equipped with a variety of transporters, such as LAT1, ENT2, RFC1, PCFT, MCT, SMCT, P-gp, ABCG2 and MRP4 and regulate drug flux between the circulating blood and the retina.
- Lipophilic cationic drugs have a great ability to increase influx transport across the BRB.
- Increasing evidence about the transport mechanisms at the BRB will help in the design of optimal drug candidates as well as the prediction of drug penetration.

This box summarizes key points contained in the article.

(RPE) cells [6,7]. It is widely believed that systemic administration is not an effective means of delivering drugs to the retina because of the blood–retinal barrier (BRB). The BRB, which consists of complex tight junctions, has a structural barrier causing restriction of nonspecific transport between the neural retina and the circulating blood [8,9]. While this particular role of the BRB is certainly beneficial to the retina, in general, the restricted drug penetration rate from the circulating blood to the retina is a major problem for retinal drug therapy. However, the BRB is not an impermeable barrier since essential nutrients are efficiently transferred to the retina from the circulating blood, and endobiotics and xenobiotics are selectively removed from the retina across the BRB [9,10]. Recent progress in BRB research has revealed that retinal endothelial cells and RPE cells express a variety of unique transporters which play a pivotal role in the influx transport of essential molecules and the efflux transport of neurotransmitter metabolites, hormones and drugs [10,11]. Retinal disposition and elimination of a therapeutic drug depends on its physicochemical properties as well as the relevant retinal anatomy and physiology. In general, due to the presence of a formidable BRB to solute transport, high doses of drugs are required to deliver therapeutic quantities of drugs to the retina following systemic administration and lead to systemic side effects. A successful design for a systemic delivery of therapeutic drugs, therefore, requires an integrated knowledge of the drugs used and the constraints associated with the systemic route of administration (**Table 1**).

The purpose of this paper is to review the factors affecting drug transport across the BRB in terms of lipophilicity and the influx and efflux transport systems in the BRB with respect to localization and substrate specificity. In addition, experimental systems for assessing the mechanisms and kinetics of influx and efflux transport will be described. In particular, the ability of these systems to predict the impact of influx and efflux transport on net retinal uptake of substrates *in vivo* will be discussed. Finally, the influence of influx and efflux transport on the systemic delivery of therapeutic drugs to the retina will be considered.

2. Blood–retinal barrier

The BRB consists of retinal capillary endothelial cells (inner BRB) and RPE cells (outer BRB) (**Figure 2**) [8,9]. The inner two-thirds of the human retina is nourished by the inner BRB and the remainder is covered by choriocapillaris via the outer BRB [12,13]. Thus, essential nutrients for photoreceptor cells are supplied across the outer BRB whereas those for neuronal cells such as ganglion cells, bipolar cells, horizontal cells, amacrine cells and Müller cells are supplied mainly across the inner BRB. Retinal capillary endothelial cells and RPE cells form tight monolayers with tight junctions, which normally prevent passive diffusion via paracellular transport of the solute across the monolayer between the circulating blood and the neural retina. For example, the influx permeability rate of D-mannitol, which is a nonpermeable paracellular marker, is more than 190-fold lower than that of D-glucose and L-arginine, which are carried by transporter-mediated transport (**Table 2**) [14-16]. Both monolayers are fully polarized since transporters which are localized in different membranes of retinal capillary endothelial cells and RPE cells have to perform influx transport of nutrimental substrates in the blood-to-retina direction and also efflux transport of metabolic waste products in the retina-to-blood direction. The luminal membrane of retinal capillary endothelial cells is in contact with blood whereas the abluminal membrane faces the retina. Similarly, the basolateral and apical membranes of RPE cells face the choroidal blood and the retina, respectively (**Figure 3**).

3. Analytical methods

Animal experimentation is an essential part in the research and development of ocular drugs and delivery systems. Although rabbits are generally used to evaluate the ocular kinetics of drugs because they have eyes similar in size to that of human eyes [17,18], the anatomy of the rabbit eye is unusual among mammals. The retinal vessels are limited to a stripe of myelinated tissue that radiates horizontally from the optic nerve head, while the remainder of the retina is avascular [19,20] and drug transport between retina and blood is mainly via the outer BRB. The retinas in humans and rats are well vascularized and have an inner BRB as well as an outer BRB [19]. *In vivo* studies using rats have indicated that radiolabeled compounds can be used as a tracer of influx and efflux transport across the BRB.

3.1 Retinal uptake index

Of the tissue sampling-single injection methods available, Alm and Törnquist first reported the retinal uptake index (RUI) method which is a modification of the brain uptake index (BUI) and the RUI has provided considerable *in vivo* information about blood-to-retina transport processes across the BRB [21]. The advantage of this approach (carotid artery injection) is that it avoids the effect of

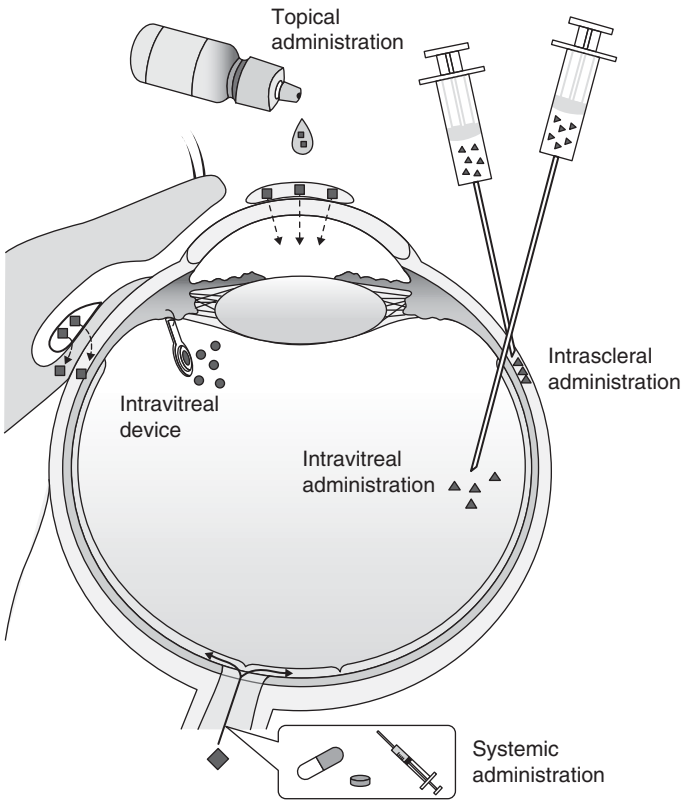

Figure 1. A schematic diagram of the eye, alongside a variety of different forms of drug administration to the posterior eye.

Table 1. Advantages and disadvantages of systemic drug delivery to treat retinal diseases.

Advantages
Easier administration than invasive methods
Patient compliance
More effective to treat retinal diseases than eye drops
Possibility of utilizing some transporter at the blood–retinal barrier
Disadvantages
Limited/variable penetration to the retina
High dose requirement
Systemic side effects

plasma-protein binding of the test substrate and allows the retinal uptake of the test substrate to be investigated in the presence of unlabelled competitor, since there is less than 5% mixing of the injected bolus (e.g., around 200 µl) with the plasma [22]. In this approach, a small bolus containing a trace dose of the [^{3}H] labeled compound of interest and a highly diffusible reference compound ([^{14}C] butanol or [^{3}H]water when a [^{14}C]test compound is studied) is injected rapidly into the carotid artery. A short time (typically 15 sec) after injection, the animal (usually a rat) is decapitated and samples of tissue and injection solution are analyzed by scintillation counting [23].

The RUI is then calculated as follows:

$$\mathrm{RUI} = ([^3\mathrm{H}]/[^{14}\mathrm{C}]\,(\mathrm{dpm\ in\ the\ retina}))/([^3\mathrm{H}]/[^{14}\mathrm{C}]\,(\mathrm{dpm\ in\ the\ injection\ solution})) \times 100$$

The RUI technique has been used primarily to determine permeation under appropriate sink conditions and is particularly useful for determining the influence of physicochemical parameters on initial retinal uptake. Using 13 compounds expected to be transported from blood to the retina by passive diffusion and with a log *n*-octanol/Ringer distribution coefficient (DC) ranging from -2.6 to 2.5, a close relationship has been established between the RUI and lipophilicity for a variety of chemical classes (**Figure 4**, open circle) [23]. Similar relationship is recently reported by Toda *et al.* [24]. The RUI technique can be used to determine whether a transporter is potentially involved in the retinal uptake of drugs across the BRB. Although compounds that do not display significant influx and efflux transport (open circle) exhibit a predictable relationship between RUI and DC, several compounds known or suspected to be substrates of influx transporters have RUI values substantially higher than those would be predicted based on their lipophilicity alone (lipophilicity trend line). On the other hand, digoxin and vincristine, known to be substrates of P-glycoprotein (P-gp/ABCB1), have RUI values substantially lower than the lipophilicity trend

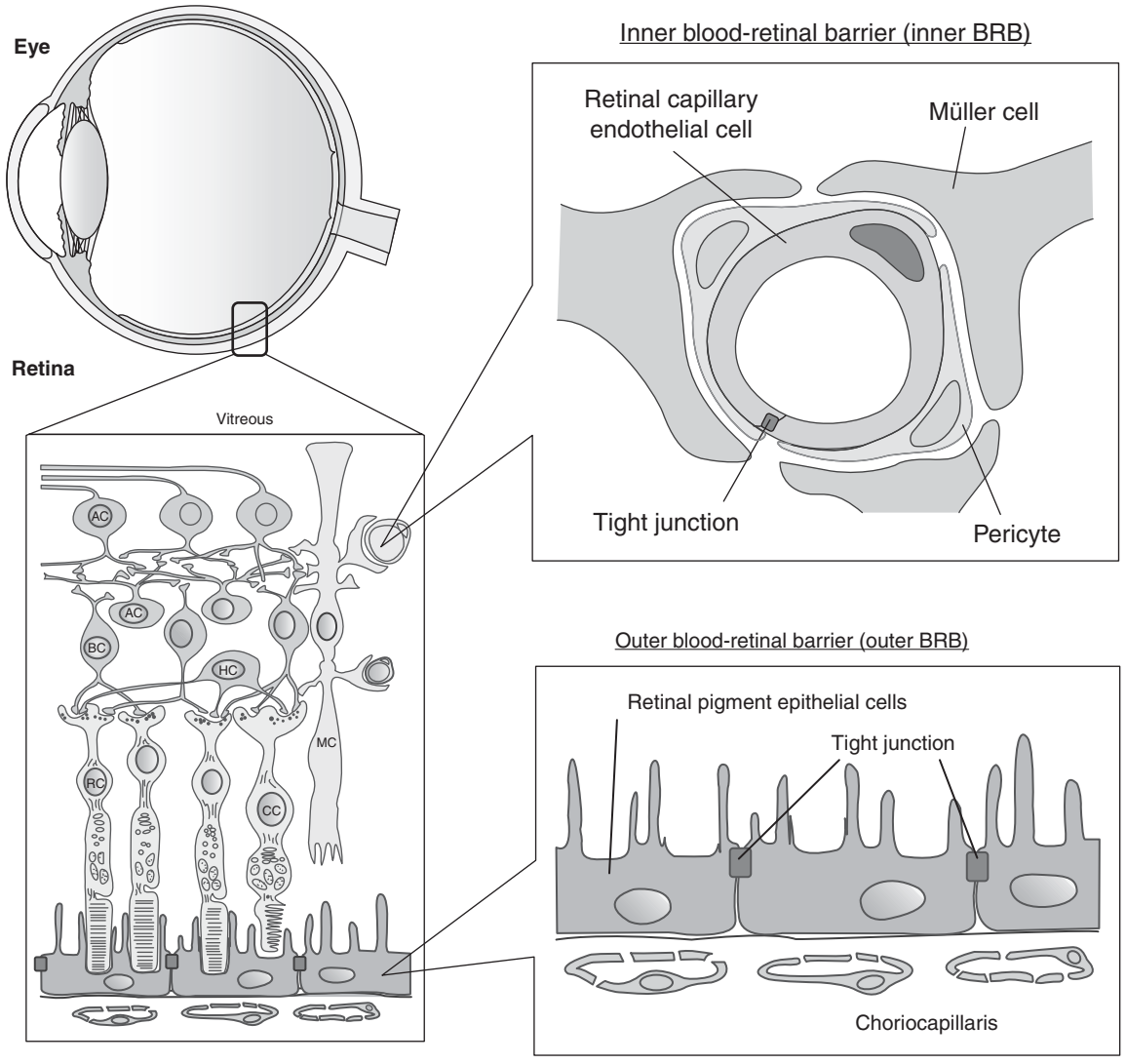

Figure 2. A schematic diagram of the blood–retinal barrier (BRB). The BRB is composed of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (RPE, outer BRB). Retinal capillary endothelial cells are surrounded by the pericyte and Müller cell foot process.

AC: Amacrine cell; BC: Bipolar cell; CC: Photoreceptor cell (cone cell); GC: Ganglion cell; HC: Horizontal cell; MC: Müller cell; RC: Photoreceptor cell (rod cell).

line [25,26]. Although warfarin was used as a model compound for passive diffusion, RUI value of warfarin is substantially lower than the lipophilicity trend line, suggesting the involvement of efflux transporter, perhaps P-gp [27]. The RUI values of [^{3}H]D-glucose, [^{3}H]L-arginine, [^{3}H]biotin, [^{3}H]L-dopa, [^{3}H]L-leucine and [^{3}H]L-phenylalanine are more than threefold greater than those predicted by the lipophilicity trend line (**Figure 4**). To confirm the involvement of a transporter for these compounds, a self-inhibition study was carried out. A significant reduction in RUI for [^{3}H]D-glucose, [^{3}H]L-arginine, [^{3}H]biotin, [^{3}H]L-dopa and [^{3}H]L-phenylalanine was obtained in the presence of D-glucose (40 mM), L-arginine (10 mM), biotin (5 mM), L-dopa (10 mM) and L-phenylalanine (0.5 mM) (**Table 2**) [23,28-30].

3.2 Integration plot analysis

The integration plot analysis allows determination of the influx permeability rate (clearance) of the test compound from the circulating blood to the retina across the BRB, even if there is only low permeability across the BRB [31,32]. The advantage of this approach is that it allows the retinal uptake of the test substrate under physiological conditions in the presence of endogenous substrates, since there is mixing of the injected bolus with the plasma [31]. In this approach, the test compound is administered intravenously and the rat is decapitated at a specified time post dosing for the collection of retina and blood samples. The apparent influx permeability rate across the BRB, CL_{BRB}, expressed as µl/(min·g retina) of the [^{3}H] or [^{14}C] labeled test compound from the circulating

Table 2. Influx transporters/transport processes at the BRB.

Transporter/transport process	Transport type	Expression and localization		Substrate	CL_{BRB} [μl/(min·g retina)]	RUI (%)	Inhibition % of control*	Plasma concentration (μM)	K_m (μM)	Ref.
		Inner BRB	Outer BRB							
SLC2A1/GLUT1	F	rt (LU, AL)	rt (BL, AP)	D-Glucose	544[§]	75.7	63 (Self 40 mM)	~ 12,500	5,000	[15,28,35]
				DHA	2440	N.D.	N.D.	10	93	[32]
SLC29A2/ENT2	F	rt (*in vitro*)		Adenosine	25.8	32.3	70 (Self 2 mM)	0.09	29	[72]
SLC7A1/CAT1	F/E	rt	rt, h (*in vitro*)	L-Arginine	118	204	30 (Self 10 mM)	170	11	[16,23,50]
SLC6A6/TAUT	Na^+	rt (*in vitro*)	m, h (*in vitro*)	Taurine	259	39.0	38 (Self 1 mM)	100 – 300	22	[30,56,118]
SLC7A5/LAT1/ (System L)	E	rt	h (*in vitro*)	L-Leucine	203	95.3	N.D.	180	14	[23,39,40]
				L-Phenylalanine	N.D.	57.6	41 (Self 0.5 mM)	80	11 – 87	[23,30,48]
				L-Dopa	N.D.	95.7	70 (Self 10 mM)	-	88	[23,41]
SLC7A11/xCT (System Xc^-)	E	rt (*in vitro*)	m, h (*in vitro*)	L-Cystine	286[¶]	N.D.	N.D.	100 – 200	10	[58,59,119]
SLC6A9/GlyT1	Na^+	rt (*in vitro*)		Glycine	8.6	21.6	71 (Self 10 mM)	200	55	[57]
SLC1A4/ASCT1/ SLC1A5/ASCT2 (System ASC)	Na^+	rt (*in vitro*)		L-Serine	49.9	N.D.	N.D.	272	98	[60]
				D-Serine	13.9	N.D.	N.D.	3	9600	[60]
SLC22A5/OCTN2	Na^+	rt (*in vitro*)		Acethyl-L-carnitine	2.3	18.0	81 (Self 2 mM)	18	26	[14]
SLC6A8/CRT	Na^+	rt (LU, AL)		Creatine	10.7	N.D.	N.D.	140 – 600	15	[38]
SLC5A6/SMVT	Na^+	rt (*in vitro*)		Biotin	5.6	31.7	63 (Self 5 mM)	0.006	146	[29]
SLC19A1/RFC1	Na^+	rt	h (AP *in vitro*)	MTF	N.D.	N.D.	N.D.	~ 0.05	5.1	[77,78]
SLC16A1/MCT1	H^+	rt (LU, AL)	rt (AP), h (AP)	L-Lactic acid	N.D.	69.6	80 (Pyruvate 10 mM)	10,000	1600	[62,63,65,67]
				Nicotinic acid	N.D.	105	48 (Self 20 mM)	0.1 – 0.4	7000	[66]
Choline transport	N.D.			Choline	271	14.5	42 (Self 10 mM)	10	6 – 100	[85,120]
Passive diffusion	-			D-Mannitol[‡]	0.6	11.6	N.D.	-	-	[14]

The influx permeability rate (CL_{BRB}) was determined using the integration plot analysis after an i.v. injection of a radiolabeled compound.

*RUI value is used as a control in the absence of an inhibitor.

[‡]D-Mannitol is a nonpermeable paracellular marker (passive diffusion).

[§]CL_{BRB} of D-glucose (544 μl/(min·g retina)) is calculated from the influx rate of D-glucose [6.8 μmol/(min·g retina)]/normal D-glucose concentration in rat plasma (12.5 mM) [15].

[¶]Indicates μl/(min·g eye).

AL: Abluminal membrane; AP: Apical membrane; BL: Basolateral membrane; BRB: Blood–retinal barrier; DHA: Dehydroascorbic acid; E: Exchanger; F: Facilitated transporter; H^+: H^+-cotransporter; h: Human; LU: Luminal membrane; m: Mouse; MTF: Methyltetrahydrofolate; Na^+: Na^+-cotransporter; N.D.: Not determined; rt: Rat.

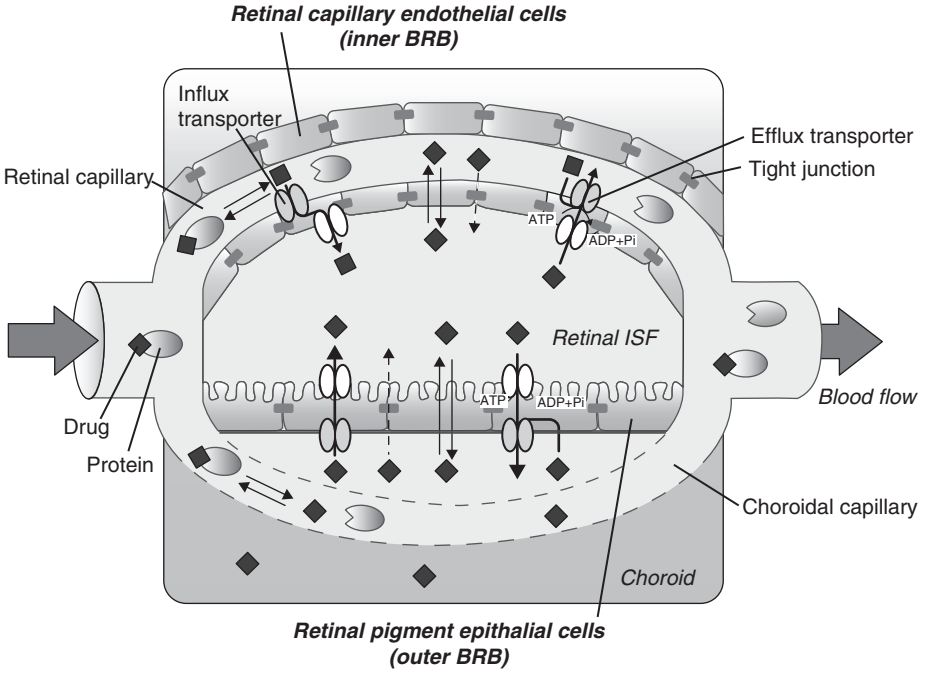

Figure 3. A schematic diagram of drug flux through the blood–retinal barrier, indicating factors and processes that determine the net retinal uptake.
ISF: Interstitial fluid.

blood to the retina across the BRB is determined by integration plot analysis. As an index of the retinal distribution characteristics of the test compound, the apparent retina-to-plasma concentration ratio (K_p) as a function of time is used. This ratio [K_p(t)] (ml/g retina) is defined as the amount of [^{3}H] or [^{14}C] per gram retina divided by that per milliliter plasma, calculated over the time period of the experiment. The CLbrb can be described by the following relationship:

$$K_p(t) = CL_{BRB} \cdot AUC(t)/C_p(t) + V_i$$

where AUC(t) (dpm·min/ml), C_p(t) (dpm/ml) and V_i (ml/g retina) represent the area under curve showing the time course of the plasma concentration of the [^{3}H] or [^{14}C]test compound from time 0 to t, the concentration of plasma [^{3}H] or [^{14}C]test compound at time t, and the rapidly equilibrated distribution volume of the [^{3}H] or [^{14}C]test compound in the retina, respectively. V_i is usually similar to the vascular volume of the retina, while CLbrb can be obtained from the initial slope of a plot of K_p(t) versus AUC(t)/C_p(t), as an integration plot.

3.3 Microdialysis analysis

Microdialysis has been recognized as a valuable tool for sampling the extracellular space of living tissue. This has been applied to sampling vitreous fluid in the rabbit and monitoring drug concentrations in the vitreous humor and neurotransmitter concentrations in the retina [17]. We have used the microdialysis technique to assess the efflux transport out of the retina [33]. In this approach, a small bolus (1.0 µl) containing a tracer dose of the [^{3}H] labeled compound of interest and a [^{14}C] labeled bulk flow marker ([^{14}C]D-mannitol or [^{3}H]D-mannitol when a [^{14}C]test compound is studied) is injected rapidly into the vitreous humor of rats. The vertical microdialysis probe (the molecular cutoff for the dialysis tubing is 50 kDa) is implanted immediately into the vitreous chamber and the [^{3}H] and [^{14}C] concentration is monitored from the dialysate (**Figure 5A**) [33]. If the compound is a substrate of the relevant efflux transporter, the elimination rate constant of the test compound is greater than that of the bulk flow marker. An example of this analysis method is shown in **Figure 5B** for [^{3}H]*p*-aminohippuric acid (PAH) used as a model compound for organic anions. After a bolus injection of [^{3}H]PAH and [^{14}C]D-mannitol, both compounds are bi-exponentially eliminated from the vitreous humor [34]. Although the initial rapid decline is almost the same for both compounds, the second decline in [^{3}H]PAH is significantly greater than that of [^{14}C]D-mannitol. The elimination rate constant of [^{3}H]PAH during the terminal phase is twofold greater than that of [^{14}C]D-mannitol, supporting the hypothesis that [^{3}H]PAH undergoes efflux transport from the vitreous humor across the BRB in addition to elimination by bulk flow [34].

4. Blood-to-retina influx transport of drugs

Membrane permeability is a key determinant of pharmacokinetic behavior, like the absorption, distribution, metabolism and excretion (ADME) of drugs. To elicit their pharmacological

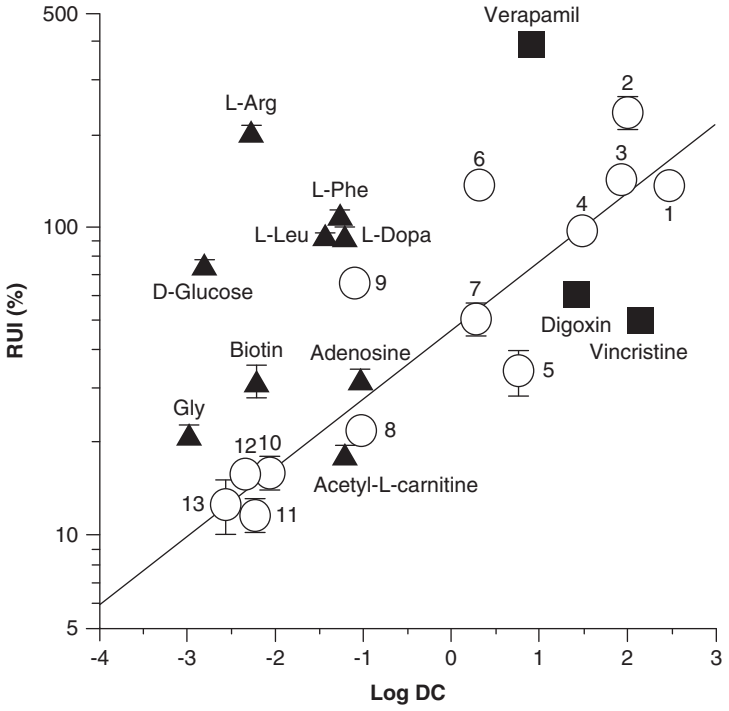

Figure 4. Relationship between the retinal uptake index (RUI) and lipophilicity [n-octanol/Ringer distribution coefficient (DC)]. Closed triangles indicate identified substrates for influx transporters; closed squares indicate identified or suspected substrates for P-glycoprotein; open circles indicate substances undergoing passive diffusion in the blood-to-retina direction; and the line indicates regression of log-transformed data indicated by the open circles (lipophilicity trend line). 1: diazepam, 2: progesterone, 3: testosterone, 4: corticosterone, 5: warfarin, 6: antipyrine, 7: valproic acid, 8: dopamine, 9: uracil, 10: creatinine, 11: D-mannitol, 12: *p*-aminohippuric acid, 13: α-methylaminoisobutyric acid.
Data taken from [23] with permission from Springer.

and therapeutic effects, a drug has to cross the BRB by passive diffusion and/or transporter-mediated transport.

4.1 Nutrients

As self-inhibition has been observed in RUI studies, nutrients have up to a 4000-fold greater influx permeability rate than that of D-mannitol, which is a nonpermeable paracellular marker (**Table 2**), implying specific transporters are involved in the blood-to-retina transfer of these compounds. Specific transporters facilitate transport of nutrients such as hexose, neutral and basic amino acids, monocarboxylic acids, nucleosides, amines and vitamins.

Facilitative glucose transporter 1 (GLUT1/SLC2A1) transports D-glucose and dehydroascorbic acid (DHA), which are the main energy source for the retina and an oxidized form of vitamin C, respectively, from the circulating blood to the retina [15,32]. GLUT1 is localized in both the luminal and abluminal membranes of the inner BRB and in both the brush-border and basolateral membranes of the outer BRB [35]. Although GLUT1 is a facilitative transporter and is expressed on both sides of the membranes, blood-to-retina transport of D-glucose and DHA is predominant rather than that in the opposite direction. This is because the expression of GLUT1 at the abluminal membrane of the inner BRB is about two- and threefold greater than that at the luminal membrane in humans and rats, respectively [35,36]. The influx permeability rate of D-glucose and DHA is 544 and 2440 µl/(min·g retina), respectively [15,32]. These figures are several times greater than that of amino acids (**Table 2**). Glycosylated neuropeptides, such as L-serinyl-β-D-glucoside analogues of Met^5enkephalin, are transported from blood to the brain via GLUT1 at the blood–brain barrier (BBB) [37], suggesting that analogs of GLUT1 substrates can be transported from the blood to the retina via GLUT1 at the BRB. However, the substrate specificity of GLUT1 seems to be very restricted and the choice of a carrier for drug delivery to the retina needs to be considered very carefully.

Creatine and biotin are essential molecules for energy homeostasis and a coenzyme in the metabolism of fatty acids and leucine, respectively, in the retina. Na^+-dependent creatine transporter (CRT/SLC6A8) and Na^+-dependent multivitamin transporter (SMVT/SLC5A6), which are expressed in retinal capillary endothelial cells, play a role in the blood-to-retina transport of creatine and biotin [29,38]. Although the influx permeability rate of creatine and biotin is 10.7 and 5.6 µl/(min·g retina), respectively, which is much lower than that of amino acids (**Table 2**), creatine and biotin are continuously transported

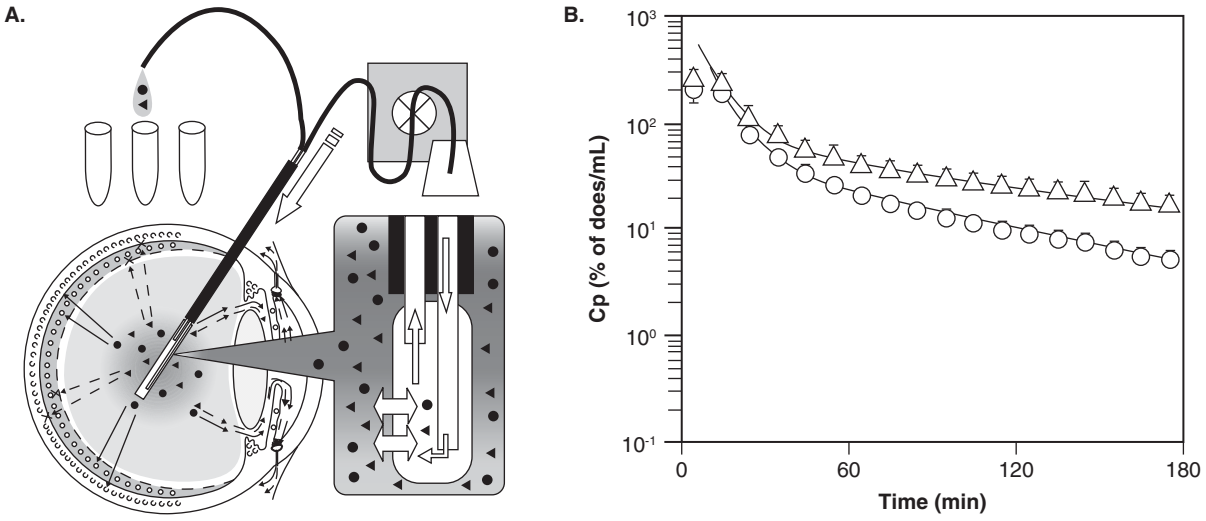

Figure 5. A schematic diagram of microdialysis monitoring of the test substrate and a bulk flow marker after vitreous bolus injection (A) and the outflow pattern of [^{3}H]*p*-aminohippuric acid (PAH) and [^{14}C]D-mannitol from the microdialysis probe **(B)**. **A**. Closed circles and triangles denote a test substrate and a bulk flow marker, respectively. Bold and dashed lines represent the tight epithelial barrier and porous tissue boundary, respectively. Bold and dashed circles represent tight and fenestrated blood vessels, respectively. **B**. Open triangles and circles represent the concentrations in the dialysate of [^{14}C]D-mannitol and [^{3}H]PAH, respectively.

(**B**) Data taken from [34] with permission from the American Society for Pharmacology and Experimental Therapeutics.

from blood to the retina to maintain their concentration there. Hence, the development of drugs that structurally mimic substrates of influx transporters is an effective strategy to increase the BRB permeability.

4.2 Amino acid-mimetic drugs

L-Leucine and L-phenylalanine are precursors of neurotransmitters and protein synthesis, and are transported from the blood to the retina via an Na^+-independent neutral amino acid transporter (system L) at the BRB (**Table 2**) [39,40]. System L also transports amino acid-mimetic drugs, such as L-dopa (amino acid precursor of dopamine), gabapentin (an analog of γ-aminobutyrate), and amino acid mustards, because of its broad substrate selectivity [41,42]. System L is encoded by L (leucine-referring)-type amino acid transporter (LAT) 1 (SLC7A5) and LAT2 (SLC7A8). LAT1 and LAT2 are unique because they require an additional protein, the heavy chain of the 4F2 cell surface antigen (CD98/SLC3A2), for functional expression [43]. Immunohistochemical analysis has revealed that LAT1 is predominantly expressed in retinal capillary endothelial cells [39]. Although RPE cells express mRNA for LAT1 and LAT2, quantitative RT-PCR and functional analyses using ARPE-19 cells suggest that LAT1 makes a major contribution to L-leucine uptake [40,44]. L-Dopa is transported across the BBB by system L, and is readily biotransformed in the brain to dopamine [41]. Many patients with Parkinson's disease have blurred vision or other visual disturbances, which are reflected in the reduced retinal dopamine concentration and delayed visual evoked potentials [45]. L-Dopa corrects these deficiencies. LAT1 transports L-dopa as a substrate with a Michaelis constant (K_m) of 88 μM [41]. Indeed, the RUI value of [^{3}H]L-dopa is similar to that of L-leucine and blood-to-retina transport of [^{3}H]L-dopa is inhibited in the presence of 10 mM L-dopa (**Figure 3** and **Table 2**) [23]. LAT1 at the BRB provides an important route for the delivery of L-dopa into the retina. Melphalan (phenylalanine mustard), which is used as cancer chemotherapy in patients with retinoblastoma, is directly injected into the vitreous humor because it is not efficiently transported from blood to the vitreous humor/retina across the BRB [46]. We investigated the potential participation of LAT1 in the retinal delivery of various amino acid-mustards as alkylating agents using a conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB cells) as an *in vitro* model of the inner BRB [47,48]. The efflux of [^{3}H] phenylalanine preloaded into TR-iBRB cells expressing LAT1 through the obligatory exchange mechanism is induced by phenylglycine mustard [39,49], but not by several other amino acid mustards tested, including melphalan [48]. This finding indicates that phenylglycine mustard is a better substrate of LAT1 than melphalan and, thus, helps in the development of a transportable alkylating agent for LAT1 at the BRB. LAT1-mediated delivery of drugs into the retina is likely to exhibit competition from its endogenous amino acid substrates *in vivo* since the normal plasma concentration of L-leucine (180 μM), L-phenylalanine (80 μM) and other neutral amino acids (52 – 220 μM) is several-fold higher than their K_m values (11 – 87 μM) [30,39]. Although these

amino acids in plasma may saturate LAT1-mediated transport at the BRB, intravenously administered [^{3}H]L-leucine is transported from blood to the retina with an influx permeability rate of 203 µl/(min.g retina) in the presence of these amino acids (**Table 2**) [39]. Therefore, L-dopa is also continuously transported from blood to the retina across the BRB. RPE cells and TR-iBRB cells express mRNA for y^+LAT1 (SLC7A7) and y^+LAT2 (SLC7A6), respectively [16,50], which transport cationic amino acids, such as L-arginine and L-lysine, in an Na^+-independent manner and neutral amino acids in an Na^+-dependent manner. Under physiologic conditions, the transport process mediated by y^+LAT1 and y^+LAT2 involves Na^+-dependent entry of neutral amino acids into cells coupled with the removal of cationic amino acids from the cells. Therefore, there may be a functional coupling between LAT1/LAT2 and y^+LAT1/y^+LAT2 in the vectorial transfer of amino acid-mimetic drugs across the inner and outer BRB, but the localization of these transporters needs to be investigated. $ATB^{0,+}$ (SLC6A14), which is expressed in RPE cells [51] but not in retinal capillary endothelial cells [14], transports a wide variety of drugs and prodrugs [52], including nitric oxide synthase inhibitors [51] and amino acid derivatives of antiviral agents, such as valacyclovir [53] and valganciclovir [54].

L-Lysine and L-arginine, which are essential and semiessential cationic amino acids, cannot be synthesized by the retina at a rate sufficient to meet the needs of retinal metabolism and protein synthesis. L-Arginine is a precursor for the generation of NO via nitric oxide synthases. Thus, the retina requires a steady and balanced supply of these cationic amino acids from the circulating blood. Na^+-independent cationic amino acid transporter 1 (CAT1/SLC7A1) is expressed at the inner and outer BRB [16]. The influx permeability rate of L-arginine is 118 µl/(min·g retina) and the blood-to-retina transport of [^{3}H]L-arginine (RUI) is inhibited in the presence of 10 mM L-arginine (**Table 2**) [16,23]. Since CAT1 also transports a variety of arginine- and lysine-based inhibitors of nitric oxide synthases [55], this transporter at the inner and outer BRB can be exploited for delivery of such compounds into the retina for the treatment of specific retinal diseases associated with the overproduction of NO (e.g., inflammation).

Several other amino acid transport systems have been identified as being involved in blood-to-retina influx transport of amino acids at the inner BRB. Na^+- and Cl^--dependent taurine transporter (TAUT/SLC6A6) [56], glycine transporter (GlyT1/SLC6A9) [57], system Xc^- which consists of xCT (SLC7A11) and 4F2hc (SLC3A2) [58,59] and Na^+-dependent transporters of system ASC (for alanine, serine and cysteine-preferring) ASCT1, 2 (SLC1A4, 1A5) [60] are expressed at the inner BRB and supply taurine, glycine, L-cystine and D/L-serine to the retina with an influx permeability rate of 8.6 – 259 µl/(min·g retina). Since the K_m values of these transporters (~ 100 µM) are several-fold lower than the physiological levels of their substrate amino acids in plasma (~ 300 µM) (**Table 2**), the blood-to-retina transport of these amino acids appears to be saturated by the endogenous amino acids under *in vivo* conditions. The role of such amino acid transporters may be important for an accurate assessment of the efficacies of exogenous amino acid-mimetic drugs in the retina in general.

4.3 Monocarboxylic drugs

Monocarboxylates, such as lactate, pyruvate and ketone bodies (β-hydroxybutyrate and acetoacetate), play a pivotal role in the cellular physiology of most mammalian cells. L-Lactic acid, in particular, is produced in huge amounts as an end-product of glycolysis. In the retina, more L-lactic acid is produced aerobically than in any other tissue [61]. Moreover, L-lactic acid appears to be required as an energy source, in addition to D-glucose, in photoreceptors [61]. The H^+-coupled monocarboxylate transporters (MCTs) belonging to the SLC16 family and the Na^+-coupled monocarboxylate transporters (SMCTs) belonging to the SLC5 family transport monocarboxylates including some monocarboxylic drugs. MCT1 (SLC16A1) is localized in both the luminal and abluminal membrane of the inner BRB [62]. RPE cells (outer BRB) express MCT1, MCT3 (SLC16A8) and SMCT1 (SLC5A8) [63,64]. MCT1 is mostly localized in the brush-border membrane and MCT3 and SMCT1 are localized in the basolateral membrane of the outer BRB [63,64]. Thus, MCT1 at the inner BRB and SMCT1 at the outer BRB can be expected to take up monocarboxylic drugs from the circulating blood. The RUI value of [^{3}H]L-lactic acid and [^{3}H]nicotinic acid is 69.5 and 105, respectively, and blood-to-retina transport of [^{3}H] L-lactic acid and [^{3}H]nicotinic acid (RUI) is inhibited in the presence of 10 mM pyruvate and 20 mM nicotinate, respectively (**Table 2**) [65,66]. MCT1 transports several monocarboxylic drugs, such as foscarnet, salicylate, benzoate and a prodrug of gabapentin [67-69]. SMCT1 recognizes benzoate, salicylate, 5-aminosalicylate and 3-bromopyruvate as substrates [70,71]. Therefore, it is capable of delivering monocarboxylic drugs into the retina via MCT1 and SMCT1 of the BRB.

4.4 Nucleoside drugs

Adenosine, which is a naturally occurring purine nucleoside, plays a number of roles in retinal neurotransmission, blood flow, vascular development and response to ischemia through cell-surface adenosine receptors. Adenosine is transported from the blood to the retina via Na^+-independent equilibrative nucleoside transporters (ENTs) at the BRB [72]. ENT1 (SLC29A1) and ENT2 (SLC29A2) transport several antiviral and anti-cancer nucleoside drugs, such as 2′, 3′-dideoxycytidine (zalcitabine, ddC), 2′, 3′-dideoxyinosine (ddI), cytarabine and gemcitabine [73,74]. ENT2 also transports 3'-azido-3'-deoxythymidine (zidovudine, AZT) [73,74]. Blood-to-retina transport of [^{3}H]adenosine (RUI) is inhibited by adenosine and thymidine but unaffected by cytidine [72]. Similar features are shown by [^{3}H]adenosine uptake by TR-iBRB cells which express ENT2 mRNA [72]. These results suggest that ENT2 most likely mediates adenosine transport at the inner BRB although the

expression of ENTs has not been examined in RPE cells. Blood-to-retina transport of adenosine is relatively low compared with amino acids with an influx permeability rate of 25.8 µl/(min·g retina) [72]. Nevertheless, the K_m value of adenosine for ENT2 is 29 µM, which is much higher than the adenosine concentration in plasma (~0.1 µM) (**Table 2**), indicating that ENT2 is not saturated with adenosine under the physiological conditions. Since the nucleoside drugs described above are substrates of ENT2, this transporter is potentially involved in the delivery of such drugs to the retina. In the chemotherapy of intraocular lymphoma, cytarabine is systemically administered in combination with methotrexate [75].

4.5 Folate analog drugs

Methotrexate is an antimetabolite and antifolate drug and acts by inhibiting the metabolism of folic acid. It is used in the treatment of intraocular lymphoma as an anticancer drug [75,76]. Folates function as cofactors for the de novo synthesis of purines, pyrimidines and some amino acids and for the conversion of homocysteine to methionine. Folate deficiency in the retina has been associated with an increased risk of nutritional amblyopia and methanol-induced retinal toxicity. Reduced folate carrier (RFC1/SLC19A1), folate receptor α and the H^+-coupled folate transporter (PCFT/SLC46A1) play a role in the uptake of folate and its analogs including methotrexate. Much of the folate in the plasma of most mammals is in the reduced form, methyltetrahydrofolate (MTF). The [^{3}H]MTF uptake by TR-iBRB cells is Na^+- and Cl^--independent and concentration-dependent with a *K*m of 5.1 µM [77]. This process is inhibited by RFC1 substrates, such as methotrexate and formyltetrahydrofolate, in a concentration-dependent manner with an IC_{50} of 8.7 and 2.8 µM, respectively [77]. RFC1 mRNA is abundantly expressed in isolated retinal capillary endothelial cells compared with PCFT mRNA [77]. Although several pieces of evidence suggest that RFC1 at the inner BRB mediates MTF transport from blood to the retina, the exact localization of RFC1 at the inner BRB presently remains unknown. In the outer BRB, folate receptor α and PCFT are expressed in the basolateral membrane and RFC1 is expressed in the brush-border membrane [78,79]. Therefore, it is suggested that folates are taken up by folate receptor α and PCFT from the blood and thereafter undergo efflux to the retina via RFC1. Methotrexate, which is used to treat eyes with intraocular lymphoma, uveitis and proliferative diabetic retinopathy [80], can be delivered into the neural retina across the BRB via the concerted actions of these three folate transport proteins.

4.6 Organic cationic drugs

Verapamil, which is a calcium channel blocker and a lipophilic cationic compound (log DC = 0.913 and pKa = 8.92), has been used in the treatment of arterial hypertension and glaucoma via systemic administration, but cardiovascular side effects posed an important problem [81,82]. The RUI value of [^{3}H]verapamil is 5.6-fold greater than the lipophilicity trend line (**Figure 4**) [23], even although verapamil is a substrate of P-gp [83]. The RUI values of [^{3}H]digoxin and [^{3}H]vincristine, which are substrates of P-gp [25,26], are lower than the lipophilicity trend line (**Figure 4**) [23]. Thus, blood-to-retina transport of verapamil is facilitated by the influx transport system at the BRB. Organic cation transporters (OCTs and OCTNs) and a transport process (choline transport) are functionally expressed at the BRB [14,84,85]. Retinal capillary endothelial cells express OCTN2 (SLC22A5) and RPE cells express OCT3 (SLC22A3) [14,84]. OCTN2 at the inner BRB mediates the blood-to-retina transport of acetyl-L-carnitine [14]. Although OCTN2 transports verapamil and several other cationic and zwitterionic drugs, including β-lactam antibiotics such as cephaloridine, tetraethylammonium, pyrilamine, quinidine and valproate as substrates [86,87], OCTN2 may not make a major contribution to the blood-to-retina transport of verapamil. This is because the influx permeability rate of acethyl-L-carnitine is quite low at 2.3 µl/(min·g retina) (**Table 2**) and the RUI value of [^{3}H]acethyl-L-carnitine is lower than the lipophilicity trend line (**Figure 4**) [23]. Although the localization of OCT3 in RPE cells remains unknown, its substrates of pharmacological significance include prazocin (α-adrenoceptor antagonist), clonidine (α-adrenoceptor agonist), cimetidine (histamine H_1 receptor antagonist), verapamil, imipramine and desipramine (antidepressants), quinine (antimalarial drug), and nicotine and methylenedioxymethamphetamine (an addictive drug) [88]. Han *et al.* produced evidence to show that there are novel organic cation transporter functions in RPE cells [89]. This transport system transports verapamil as a substrate with a K_m of 7.2 µM and recognizes diphenhydramine, pyrilamine, quinidine and quinacrine [89]. Zhang *et al.* found that brimonidine (an α2-adrenergic agonist approved for the treatment of open-angle glaucoma, pKa = 7.4) is transported in a carrier-mediated manner in RPE cells [90]. Brimonidine uptake by RPE cells takes place in a pH-sensitive, temperature-dependent and concentration-dependent manner with a K_m of 51 µM, and is inhibited by verapamil and quinidine [90]. Since systemically administered brimonidine can reach the back of the eye at concentrations sufficient to activate α2-adrenergic receptors [91], the novel organic cation transporter in RPE cells regulates the brimonidine concentration in the retina. Memantine hydrochloride, which is currently used for the treatment of Alzheimer's disease, is a lipophilic cationic compound (log DC = 3.28 and pKa = 10.4) [92]. Its neuroprotective properties suggest that it may be beneficial for the treatment of glaucoma. The retinal membrane concentration of memantine was found to be 108 ng/mL after oral administration to rabbits (2 mg/kg for 7 days) [93]. In glaucoma patients, neuroprotective effects are obtained by memantine treatment (orally 20 mg/day for 6 months) [94]. Although there is no information from BRB transport studies, it may be that a novel organic cation transporter at the BRB regulates the memantine concentration in the retina. Chloramphenicol (antibiotic, log DC = 1.1 and pKa = 11.0)

appears to be transported into the vitreous/retina to a significant extent after systemic administration [93,95]. A novel organic cation transporter/organic cation transporters responsible for the transfer of organic cations across the BRB have a great potential for the delivery of various organic cationic drugs into the retina although the molecular identity of the transporter remains to be established.

5. Retina-to-blood efflux transport of drugs

5.1 ABC transporters

Efflux transport at the BRB serves to limit the presentation of systemically available substrates to the retina. Accordingly, a relevant efflux transport system will be the primary determinant of the substrate concentration in retinal interstitial fluid. Efflux of xenobiotics, including drugs which are taken up from the circulating blood, can be initiated at the luminal and basolateral membrane of the inner and outer BRB, respectively, as in the case of ATP-binding cassette (ABC) transporters (**Figure 3**). P-gp is an ATP-dependent efflux pump which mediates a rapid removal of a wide variety of chemotherapeutic agents and lipophilic compounds. P-gp is encoded in humans by the *MDR1* gene and in rodents by the *mdr 1a* and *mdr 1b* genes and is located in the luminal membrane of the inner BRB [12] and mostly in the basolateral membrane of the outer BRB [96]. As shown in **Figure 4**, RUI values of [^{3}H]digoxin and [^{3}H]vincristine are lower than the lipophilicity trend line. In addition, blood-to-retina transport of [^{3}H]digoxin (RUI) is increased in the presence of 5 mM verapamil which is an inhibitor of P-gp [23]. Cyclosporin A, which is a substrate of P-gp, was not detected in the intraocular tissues of cyclosporin A-treated rabbits [97], rats [98] and humans [99], although a significant level of cyclosporin A was detected in plasma. These pieces of evidence indicate that P-gp at the BRB plays an important role in protecting the retina from xenobiotics and, therefore, hinders the retinal transfer of drugs. In addition to P-gp, several multidrug resistance-associated proteins (MRP/ABCC) and breast cancer resistance protein (BCRP/MXR/ABCP/ABCG2) are expressed at the inner and outer BRB [100,101]. MRPs play a role in transporting anionic compounds, such as glucuronic acid conjugates and glutathione conjugates. ABCG2 prefers not only drugs (e.g., mitoxantrone and doxorubicin) but also photosensitive toxins, including pheophorbide a, a chlorophyll-derived dietary phototoxin related to porphyrin. The transcript levels of MRPs at the inner BRB have been quantified in isolated mouse [100] and rat retinal capillary endothelial cells [9]. MRP4 (ABCC4) was shown to be most highly expressed at the transcript level and MRP3 (ABCC3) (mouse) and MRP6 (ABCC6) (mouse and rat) were also expressed at lower levels [9,100]. Consistent with these transcript data, it has been recently demonstrated that MRP4 protein is localized on the luminal membrane of the inner BRB in mice [102]. Among the six genes coding for MRPs, MRP1, MRP4, and MRP5 are expressed in human RPE cells [101]. Although localization of these MRPs is still unknown, functional experiments suggest that MRPs are located on the basolateral membrane of RPE cells [103]. ABCG2 is localized on the luminal membrane of the inner BRB in rats [104] and may not be expressed in RPE cells. Regarding the retina-to-blood transport of organic anions, the first step is the uptake of these compounds by transporters on the abluminal and brush-border membranes and subsequently these compounds undergo efflux to the circulating blood via MRPs and ABCG2 on the luminal and basolateral membrane of the inner and outer BRB, respectively. Of the transporters on the abluminal and brush-border membrane at the inner and outer BRB, the organic anion transporting polypeptide (OATP/SLCO/SLC21A) and organic anion transporter (OAT/SLC22A) family take part in the uptake of organic anions.

5.2 Organic anionic drugs

The distribution of β-lactam antibiotics in the vitreous humor/retina after systemic administration is limited, resulting in reduced efficacy in the treatment of bacterial endophthalmitis [105]. Some β-lactam antibiotics, such as benzylpenicillin (PCG), are substrates of organic anion transporter (OAT) 3 (SLC22A8) [106]. 6-Mercaptopurine (6-MP) is frequently used for cancer chemotherapy in patients with childhood acute lymphoblastic leukemia. Relapse of childhood acute lymphoblastic leukemia involving the eye is a rare phenomenon but poses a challenging problem [107]. This is probably due to the restricted distribution of 6-MP in the eye. One possible factor involved in the restricted drug distribution in the retina/eye is the retina-to-blood efflux transport of such anionic drugs across the BRB. In addition to PAH (**Figure 5B**) PCG and 6-MP were bi-exponentially eliminated from the vitreous humor after bolus injection into the vitreous humor of the rat eye [34]. The elimination rate constant of [^{3}H]PCG, and [^{14}C]6-MP during the terminal phase was about twofold greater than that of D-mannitol. The efflux transport of [^{3}H]PAH, [^{3}H]PCG and [^{14}C]6-MP was reduced in the retina in the presence of probenecid, PAH and PCG, relatively specific substrates of OAT3 [106], but not in the presence of digoxin which is a specific inhibitor of organic anion transporting polypeptide (oatp) 1a4 (Slco1a4/oatp2) (**Table 3**) [108]. OAT3 is localized on the abluminal membrane of retinal capillary endothelial cells [34]. MRP4 is known to transport PAH, 6-MP and β-lactam antibiotics as substrates [109]. Thus, OAT3 and MRP4 are involved in the uptake from interstitial fluid and excretion of PAH, PCG and 6-MP across the abluminal and luminal membranes of retinal capillary endothelial cells, respectively. OAT3 and MRP4 contribute to the efflux transport of PAH, PCG and 6-MP from vitreous humor/retina into blood across the inner BRB.

[^{3}H]Estradiol 17-β glucuronide (E17βG) and [^{3}H]dehydroepiandrosterone sulfate (DHEAS), glucuronic acid and sulfate conjugates for a neuroactive steroid, respectively, are

Table 3. Effect of several inhibitors on efflux transport of organic anions across the BRB.

Inhibitors	Concentration	Percentage of control				
		[^{3}H]E17βG	[^{3}H]DHEAS	[^{3}H]PAH	[^{3}H]PCG	[^{14}C]6-MP
Control		100 ± 22	100 ± 10	100 ± 5	100 ± 8	100 ± 3
PAH	0.7 mM	95.8 ± 14.0	83.9 ± 19.0	N.D.	N.D.	N.D.
	1.5 mM	N.D.	44.7 ± 21.8*	38.9 ± 15.5**	81.3 ± 7.5	83.2 ± 7.4
	3.3 mM	N.D.	N.D.	N.D.	51.4 ± 13.2**	70.5 ± 10.4*
PCG	1.6 mM	N.D.	N.D.	65.6 ± 7.6*	62.1 ± 6.7**	50.4 ± 8.9**
	3.3 mM	N.D.	N.D.	48.9 ± 14.0**	31.5 ± 2.9**	N.D.
	8.3 mM	N.D.	N.D.	1.26 ± 7.90**	22.1 ± 7.5**	N.D.
Probenecid	1.0 mM	0.35 ± 0.10**	32.9 ± 9.8**	53.1 ± 8.8**	64.7 ± 10.2**	58.2 ± 6.9**
BSP	0.6 mM	6.17 ± 5.80**	12.0 ± 11.0**	65.7 ± 5.0*	N.D.	N.D.
Digoxin	0.35 μM	17.0 ± 8.6**	24.0 ± 12.3**	101 ± 8	99.2 ± 11.0	101 ± 10
E17βG	0.3 mM	9.50 ± 5.56**	26.7 ± 16.8**	N.D.	N.D.	N.D.
DHEAS	0.1 mM	28.7 ± 8.4**	32.3 ± 8.4**	N.D.	N.D.	N.D.

Each inhibitor was perfused in the microdialysis probe. Each item of data represents the mean ± S.E.M. (n = 3 – 16).

Data taken from [33,34] with permission from Elsevier and the American Society for Pharmacology and Experimental Therapeutics, respectively.

*p < 0.05, **p < 0.01, significantly different from control.

BSP: Sulfobromophthalein; DHEAS: Dehydroepiandrosterone sulfate; E17βG: Estradiol 17-β glucuronide; N.D.: Not determined; PAH: *p*-aminohippuric acid; PCG: Benzylpenicillin, 6-MP: 6-mercaptopurine.

bi-exponentially eliminated from the vitreous humor following a bolus injection into the vitreous humor of the rat eye [33]. The elimination rate constant of [^{3}H]E17βG and [^{3}H]DHEAS during the terminal phase was twofold greater than that of D-mannitol and it was significantly inhibited by organic anions, including digoxin, a specific substrate of oatp1a4 [108]. DHEAS efflux was also inhibited in the presence of PAH, which is a relatively specific substrate of OAT3 (**Table 3**) [106]. Oatp1a4 is expressed in rat retinal capillary endothelial cells and RPE cells [110]. Moreover, oatp1a4 and 1c1 (Slco1c1/oatp14) mRNA are predominantly expressed in isolated rat retinal capillary endothelial cells [111]. Oatp1c1 transports E17βG as is the case with oatp1a4 whereas oatp1c1 does not have a high affinity for digoxin [112]. MRP4 is known to transport E17βG and DHEAS as substrates [113]. Thus, E17βG and DHEAS are taken up by oatp1a4 and/or OAT3 on the abluminal and brush-border membrane of retinal capillary endothelial cells and RPE cells and undergo efflux from the cells to blood, most likely via MRP4.

Many clinically important drugs, including antibiotics, anticancer drugs, anti-HIV drugs and anti-inflammatory agents, are organic anions and, therefore, such drugs are actively removed from the retina across the BRB, thus preventing accumulation of these drugs in the retina at therapeutically effective concentrations. This hurdle might be overcome, however, if specific inhibitors of organic anion transporters are administered along with the drugs. Sunkara *et al.* demonstrated that pre-administration of probenecid, an organic anion transport inhibitor, increases the retinal concentration of N-4-benzoylaminophenylsulfonylglycine, a novel anionic aldose reductase inhibitor [114]. It is likely that inhibition of drug efflux transporters leads to an increased distribution of drug to the retina by lowering its retina-to-blood efflux transport. However, this approach needs to consider changes in drug distribution in peripheral tissues and the brain since efflux transporters are also expressed in peripheral tissues and the BBB [115].

6. Conclusions

The BRB, which consists of a complex of tight junctions, primarily restricts nonspecific transport of hydrophilic substances from blood to the neural retina and contains a variety of transporters. In addition to a structural barrier, ABC transporters such as P-gp, ABCG2 and MRP4, which are localized in the luminal and/or basolateral membrane of the BRB, limit the distribution of several lipophilic and anionic drugs. However, LAT1 and other amino acid transporters are expressed at the inner and outer BRB and transport amino acid-mimetic drugs, such as L-dopa and nitric oxide synthase inhibitors. Other influx transporters, such as ENT2, RFC1/flolate receptor α/PCFT and MCT/SMCT, play a role in the blood-to-retina transport of substrate drugs as well as physiological substrates. Although a novel organic cation transporter has not yet been identified, it is responsible for the transfer of lipophilic cationic drugs across the BRB and has great potential for the delivery of various organic cationic drugs into the retina. These influx and efflux transporters and transport mechanisms at the BRB can be exploited for the design of optimal retinal drug delivery systems. Although we have seen remarkable progress in recent years in the identification and characterization of the transporters at the BBB, our understanding of the BRB transport systems is still limited because information regarding transporters at the BRB is derived from studies in rodents. Establishing a quantitative atlas of membrane protein expression of transporters in human retinal capillary endothelial cells and RPE cells using liquid chromatography-tandem mass spectrometry

(LC/MS/MS) [116] will help us to predict drug transport to the human retina across the BRB. Due to the regulation of transporters at the BRB under disease conditions remains largely unknown, precise quantification of their expressional alteration in retinal diseases will provide information about the detailed pathological features of retinal diseases and strategies for therapy of retinal diseases.

7. Expert opinion

The BRB forms a complex of tight junctions of retinal capillary endothelial cells and RPE cells to restrict nonspecific transport between the circulating blood and the neural retina. OATP, OAT, MRP and ABCG2 at the BRB appear to be involved in the efflux transport of anionic drugs. Therefore, hydrophilic anionic drugs cannot be easily delivered to the retina from the circulating blood. Lipophilic drugs prefer the transcellular transport at the BRB as shown in **Figure 4**, although this benefit may be offset by an increase in the total clearance and binding to plasma proteins. Lipophilic drugs distribute to other tissues as well as the retina. Transporter-mediated drug transport at the BRB offers great advantages over passive diffusion. Therefore, it is important to design and select optimal drug candidates by taking into the account the fact that drugs should be recognized by influx transporters and also that efflux transporters at the BRB should be avoided. Although verapamil is a substrate of P-gp [83], blood-to-retina transport of verapamil is substantially higher than the lipophilicity trend line (**Figure 4**). This is due to the fact that a novel organic cation transporter may facilitate the transfer of verapamil across the BRB. Although lipophilic cationic drugs are known to be transported to the brain across the BBB [117], verapamil transport to the retina is substantially higher than to the brain [23]. Therefore, lipophilic cationic drugs have a great ability to increase influx transport across the BRB. Another reason is that P-gp is less active at the BRB than the BBB, since transport of the P-gp substrate, digoxin, to the retina is also higher than that to the brain [23].

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Declaration of interest

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