Pharmaceutical Research, Vol. 26, No. 9, September 2009 (© 2009)
DOI: 10.1007/s11095-009-9930-2

Expert Review

Inner Blood-Retinal Barrier Transporters: Role of Retinal Drug Delivery

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Received April 30, 2009; accepted June 16, 2009; published online July 1 2009

Abstract. The inner blood-retinal barrier (inner BRB) forms complex tight junctions of retinal capillary endothelial cells to prevent the free diffusion of substances between the circulating blood and the neural retina. Thus, understanding of the inner BRB transport mechanisms could provide a basis for the development of strategies for drug delivery to the retina. Recent progress in inner BRB research has revealed that retinal endothelial cells express a variety of unique transporters which play a role in the influx transport of essential molecules and the efflux transport of xenobiotics. In this review we focus on the transport mechanism at the inner BRB in relation to its importance in influencing the inner BRB permeability of drugs.

KEY WORDS: carrier-mediated transport; efflux transport; influx transport; inner blood-retinal barrier; retinal capillary endothelial cells; transporter.

INTRODUCTION

Retinal diseases, including age-related macular degeneration, diabetic retinopathy, glaucoma, and retinitis pigmentosa, are accompanied by severe vision loss and impose a serious socioeconomic burden. The treatment of these retinal diseases is challenging, since the ocular barriers that effectively protect the eye from foreign materials also hinder the efficient absorption of pharmaceuticals. Although topical delivery (eye drops) has been used clinically to treat ocular diseases, it is ineffective in producing therapeutic concentrations in the posterior segment tissues because of the longer diffusional distance and counterdirectional intraocular convection from the ciliary body to Schlemm's canal (1). Although intravitreal delivery of drugs by means of implants and injections is able to maintain therapeutic concentrations in the posterior segment tissues (2), it has been associated with serious side effects such as postoperative endophthalmitis, hemorrhage and retinal detachment (3).

The retina has a unique position with regard to pharmacokinetics in that the blood-retinal barrier (BRB) separates the retina from the circulating blood. The BRB, which forms complex tight junctions of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (outer BRB), restricts nonspecific transport between the neural retina and the circulating blood (Fig. 1) (4,5). Therefore, in general, systemic drug administration is not suitable for the treatment of retinal diseases because of poor drug permeability across the BRB. However, the BRB efficiently supplies nutrients to the retina and removes endobiotics and xenobiotics from the retina to maintain a constant milieu in the neural retina (6). Recent progress in inner BRB research has revealed that retinal capillary endothelial cells express a variety of unique transporters which play a key role in the influx transport of essential molecules and the efflux transport of neurotransmitter metabolites, toxins, and drugs. In the past, transporters have been considered to make a limited contribution to pharmacokinetics, but this paradigm has changed and it is now known that many transporters play an important role in pharmacokinetics (7–9). A better understanding of the molecular basis of transport functions at the inner BRB will provide a basis for developing strategies aimed at drug delivery to the retina. This review will focus on the molecular mechanism of the carrier-mediated transport systems at the inner BRB in relation to their importance in influencing the inner BRB permeability of drugs.

INFLUX TRANSPORTERS AT THE INNER BLOOD-RETINAL BARRIER

In the retina, neuronal cells, including photoreceptor cells, require a large amount of metabolic energy for phototransduction and neurotransduction (10,11). Since metabolic substrates, such as D-glucose and amino acids, are hydrophilic substances, the inner BRB limits passive diffusion of these molecules. Therefore, one of the most important roles of transporters at the inner BRB is to meet the high energy demand in the retina by supplying a source of energy from the circulating blood. The inner BRB influx transporters supply hydrophilic nutrients, such as D-glucose, amino acids, vitamins, and nucleosides, to the retina. The *in vivo* blood-to-retina transport of a test compound across the BRB is evaluated by integration plot analysis after intravenous injection of the radiolabeled compound in rats

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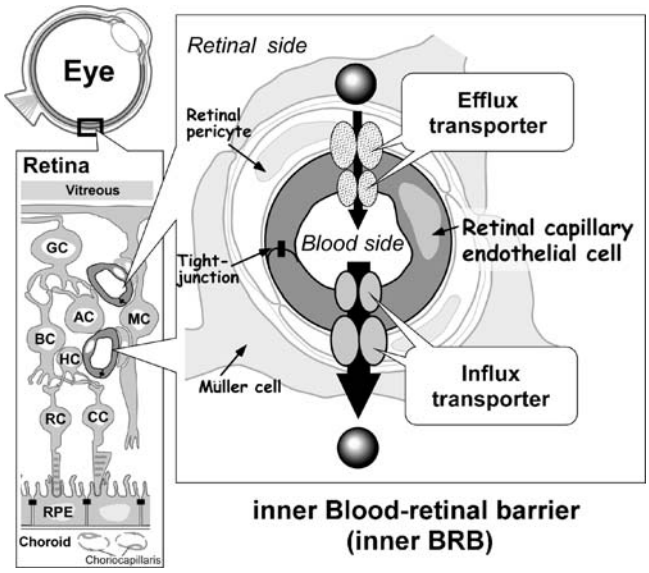

Fig. 1. Schematic diagram of the relationship between blood-to-retina influx and retina-to-blood efflux transport via influx and efflux transporters, respectively, at the inner blood-retinal barrier. The inner blood-retinal barrier forms complex tight-junctions of retinal capillary endothelial cells which are surrounded by the pericyte and Müller cell foot process. *GC* ganglion cell; *AC* amacrine cell; *BC* bipolar cell; *HC* horizontal cell; *MC* Müller cell; *RC* photoreceptor cell (*rod cell*); *CC* photoreceptor cell (*cone cell*); *RPE* retinal pigment epithelial cell.

(Table I) (12). This approach allows determination of the apparent retinal uptake of a test compound, or influx permeability rate (μL min^{-1} g^{-1} retina), even if there is only low permeability across the BRB. The influx permeability rates differ for carrier-mediated transport and passive diffusion.

TRANSPORTER-MEDIATED INFLUX TRANSPORT

GLUT1

D-Glucose, which is the main energy source for the retina, is transported from the circulating blood to the retina via facilitative glucose transporter 1 (GLUT1/SLC2A1). GLUT1 transports hexoses and dehydroascorbic acid (DHA), which is an oxidized form of vitamin C, and 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 (13–15). The expression of GLUT1 at the abluminal membrane of the inner BRB is approximately 2- and 3-fold greater than that at the luminal membrane in humans and rats, respectively (14,15). This asymmetrical distribution of GLUT1 at the inner BRB suggests that D-glucose transport is limited at the blood-to-luminal rather than the abluminal-to-interstitial interface. The influx permeability rate of D-glucose is 544 μL min^{-1} g^{-1} retina which is calculated from the influx rate of D-glucose (6.8 μmol min^{-1} g^{-1} retina) and the normal D-glucose concentration in the rat plasma (12.5 mmol L^{-1}) (Table I) (16).

Table I. Parameters of the Blood-to-Retina Influx Transport System at the inner BRB

	Plasma concentration (μM)	Km (μM)	Influx permeability rate (μL min^{-1} g^{-1} retina)	References
DHA	10	93	2,440	17
D-Glucose	~12,500	5,000	544[a]	16
L-Cystine	100–200	10	286[b]	25
Choline	10	6–100	271	49
Taurine	100–300	22	259	24
L-Leucine	180	14	203	23
Adenosine	0.09	29	25.8	27
Creatine	50–200	15	10.7	26
D-Mannitol	–	–	0.75	19
Sucrose	–	–	0.27	19

The influx permeability rate was determined by integration plot analysis after intravenous injection of radiolabeled compound
[a] Influx permeability rate of D-glucose [544 (μL min^{-1} g^{-1} retina)] is calculated from the influx rate of D-glucose (6.8 μmol min^{-1} g^{-1} retina)/normal D-glucose concentration in rat plasma (12.5 mmol L^{-1}) (16)
[b] indicates mL^{-1} g^{-1} eye. DHA: dehydroascorbic acid

DHA has the highest influx permeability rate with 2,440 μL min^{-1} g^{-1} retina (Table I). DHA transport is mediated via GLUT1 at the inner and outer BRB and it is converted to ascorbic acid in the retina (17,18). In contrast, sucrose and D-mannitol have the lowest influx permeability rates with 0.27 and 0.75 μL min^{-1} g^{-1} retina, respectively (Table I) (19). Both compounds are used as non-permeable paracellular markers and are transported by passive diffusion. The difference between DHA and sucrose is about 10,000-fold. Although GLUT1 is a facilitative glucose transporter with a Michaelis-Menten (Km) constant of 5–7.8 mM for D-glucose (16,20), DHA uptake via GLUT1 is competitively inhibited by D-glucose, and the normal plasma D-glucose concentration in most mammals is ~5 mM. Therefore, DHA transport via GLUT1 across the BRB does not exhibit complete inhibition under normal conditions. On the other hand, DHA transport from the blood to the retina decreases with increasing blood D-glucose concentration under diabetic conditions because of inhibition of DHA uptake by GLUT1 at the BRB (21). In the brain, the L-serinyl-β-D-glucoside analogues of Met^5enkephalin are transported from blood to the brain via GLUT1 at the blood-brain barrier (BBB) (22). This suggests that analogues 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.

TAUT

The influx permeability rates of amino acids, such as L-leucine, taurine, and L-cystine, are 200–290 μL min^{-1} g^{-1} retina/eye and more than 10-fold greater than that of creatine and adenosine (Table I) (23–27). Taurine is the most abundant free amino acid in the retina (12 mM in rats) and accounts for more than 50% of the free amino acid content of the rat retina (28). Taurine is physiologically important in the retina, since taurine depletion in the retina interferes with the ability of the cells to maintain their volume under altered osmotic conditions and antioxidant activity, leading to retinal dysfunction (29). We recently found that TAUT (SLC6A6) transports γ-aminobutyric acid (GABA) as a substrate with a lower affinity than taurine (30). Although the physiological role of TAUT in GABA transport in the retina is not fully understood, TAUT most likely mediates taurine transport from the circulating blood to the retina (24).

System L and Xc^-

The system L and Xc^- consist of respective LAT1 (SLC7A5) and xCT (SLC7A11) and an additional subunit protein of the heavy chain of 4F2 cell surface antigen (4F2hc/SLC3A2) and are involved in the transport amino acids (31,32). LAT1 transports branched-chain and aromatic amino acids which are essential amino acids as a precursor for neurotransmitters and protein synthesis (31,33). To protect the retina against light-induced oxidative stress and maintain intracellular antioxidants, such as gluthathione (GSH) and vitamin C, at an appropriate level (34,35), transport of L-cyst(e)ine (one of the constituent amino acids for GSH) and vitamin C into the retina is critical for the health of the retina. xCT is expressed at the inner BRB and transports L-cystine, which is also one of the rate-limiting precursors of GSH synthesis (25,36). An immunohistochemical study showed that LAT1 is expressed at the inner BRB (23). LAT1 is potentially important for drug delivery into the retina. L-Dopa is transported across the BBB by system L, and is readily biotransformed in the brain to dopamine (37). 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 (38). L-Dopa administration has been reported to reduce these delayed visual evoked potentials in Parkinson's disease (39). Amino acid mimetic-drugs, such as melphalan (phenylalanine-mustard) and gabapentin, seem to be transported as substrates of LAT1 (40). LAT1 is an amino acid exchange transporter which makes it possible to evaluate whether compounds accepted by the binding site of LAT1 are transported or not by examining their ability to induce the efflux of loaded radiolabeled substrates (41). We have investigated the transport screening of amino acid-mustards using a conditionally immortalized rat retinal capillary endothelial cell line (TR-iBRB) which expresses LAT1 (23,42). We synthesized five amino acid-mustards: tyrosine-mustard, phenylglycine-mustard, alanine-mustard, ornithine-mustard, and lysine-mustard (43). Melphalan failed to induce the efflux of loaded [^{3}H]L-phenylalanine (L-Phe) as is the case with L-arginine which is not a substrate for LAT1. In addition to melphalan, tyrosine-mustard, alanine-mustard, ornithine-mustard, and lysine-mustard did not induce any significant efflux, suggesting that these compounds are poor substrates of LAT1. In contrast, phenylglycine-mustard induced the efflux of loaded [^{3}H]L-Phe as is the case with transportable substrates of LAT1, such as L-phenylalanine, L-leucine, and 2-aminobicyclo-(2,2,1)-heptane-2-carboylic acid. This suggests that phenylglycine-mustard is a more potent substrate of LAT1 than melphalan. However, further studies are needed to determine the anti-tumor activities of phenylglycine-mustard.

ENT2

Adenosine is an important intercellular signaling molecule, and it plays a number of roles in retinal neurotransmission, blood flow, vascular development, and response to ischemia through cell-surface adenosine receptors (44,45). TR-iBRB cells exhibit an Na^+-independent, nitrobenzylmercaptopurine riboside-insensitive, and concentration-dependent [^{3}H] adenosine uptake with a Km of 29 μM, suggesting that ENT2 (SLC29A2) is involved in [^{3}H]adenosine uptake by TR-iBRB cells (27). Adenosine, inosine, uridine, and thymidine inhibit this process by more than 60%, while cytidine inhibits it by 30%. Quantitative real-time PCR has shown that ENT2 mRNA is predominantly expressed in TR-iBRB cells. A retinal uptake index (RUI) study has demonstrated that [^{3}H]adenosine uptake from the circulating blood to the retina is inhibited by adenosine and thymidine but unaffected by cytidine, which is similar to the results obtained in TR-iBRB cells (27). These results suggest that ENT2 most likely mediates adenosine transport at the inner BRB and it is expected to have the ability to modulate retinal functions by regulating the adenosine concentration in retinal interstitial fluid. Although the influx permeability rate of

adenosine is 25.8 μL min^{-1} g^{-1} retina, which is about 10-fold lower than that of amino acids (Table I), the Km of adenosine uptake by TR-iBRB cells was found to be 29 μM, while the plasma concentration of adenosine is 0.09 μM (46). This is because ENT2 at the inner BRB is not saturated by plasma adenosine. ENT2 accepts some antiviral or anticancer nucleoside drugs, like 3′-azido-3′-deoxythymidine (AZT), 2′ 3′-dideoxycytidine (ddC), 2′ 3′-dideoxyinosine (ddI), cladribine, cytarabine, fludarabine, gemcitabine, and capecitabine, as preferred substrates (47,48). This has led to the hypothesis that ENT2 at the inner BRB could be a potential route for delivering nucleoside drugs from the circulating blood to the retina.

Other Transporters

Various other transport systems and processes have been identified as being involved in blood-to-retina influx transport at the inner BRB (Tables I and II). Although the choline transporter has not been identified yet at the inner BRB, the influx permeability rate is as high as 271 μL min^{-1} g^{-1} retina (Table I) (49). Choline plays an essential role in constituents of the cell membrane, such as phosphatidylcholine and a precursor of acetylcholine which is used exclusively as a neurotransmitter by starburst amacrine cells (50,51). The [^{3}H] choline uptake by TR-iBRB cells is Na^+-independent, potential-dependent, and concentration-dependent with Km of 6.4 and 100 μM, and is inhibited by several organic cations but not tetraethylammonium (49). There are high- and low-affinity transport processes for choline in TR-iBRB cells. Although Na^+-independent organic cation transporter (OCT) 1 (SLC22A1) and 2 (SLC22A2) are known to mediate choline as a substrate with a comparably low affinity (~400 μM) (52), OCT1 and 2 mRNAs in TR-iBRB cells were not detected by RT-PCR analysis (49). Rat choline transporter-like protein (CTL) 1 (SLC44A1) mRNA is expressed in TR-iBRB cells. However, the reduction in CTL1 mRNA by CTL1 siRNA hardly inhibited the uptake of choline in TR-iBRB cells, suggesting that CTL1 is not responsible for choline transport in TR-iBRB cells (49). The choline transport system(s) has not been identified at a molecular level as is the case for those at the BBB (53,54). The role of such a choline transport process may become important when accurately assessing the efficacy of exogenous cationic drugs in the retina in general (55).

Creatine plays a vital role in the storage and transmission of phosphate-bound energy due to the conversion of creatine to phosphocreatine (56). In order to maintain a high concentration of creatine and ATP homeostasis in the retina, the transport of creatine from the circulating blood to the retina across the BRB is important in the treatment of gyrate atrophy (GA) of the choroid and retina with hyperornithinemia. This results in high ornithine and low creatine concentrations in the body fluids and leads to chorioretinal degeneration (57). It has been shown that S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase, a key enzyme for creatine synthesis, is preferentially expressed and creatine is biosynthesized in the Müller glia of the retina (58). An *in vivo* intravenous administration study has demonstrated that [^{14}C]creatine is transported from the blood to the retina against the creatine concentration gradient that exists between the retina and blood (influx permeability rate: 10.7 μL min^{-1} g^{-1} retina) (Table I) (26). [^{14}C]Creatine uptake by TR-iBRB cells took place in an Na^+- and Cl^--dependent manner and was inhibited by creatine transporter (CRT/SLC6A8) inhibitors. CRT mRNA and protein were expressed in the

Table II. Representative Transporters and Transport Processes at the inner BRB

Transporters and transport processes	Substrates	Expression and functions		References
		In vivo	*In vitro*	
Blood-to-retina influx transport				
GLUT1	D-Glucose/DHA	+	TR-iBRB	14,17,42
MCT1	L-Lactic acid	+	TR-iBRB	63–65
CRT	Creatine	+	TR-iBRB	26
LAT1/4F2hc (system L)	Large neutral amino acids	+	TR-iBRB	23
XCT/4F2hc (system X_c^-)	L-Cystine	Integration plot	TR-iBRB	25,36
TAUT	Taurine/GABA	Integration plot	TR-iBRB	24, 30
System y^+	L-Arginine	RUI	TR-iBRB	72
ENT2	Nucleosides	RUI	TR-iBRB	27
RFC1	MTF	Ex vivo	TR-iBRB	70
Choline transport process	Choline	Integration plot	TR-iBRB	49
SR-BI	α-Tocopherol	+	TR-iBRB	77
Retina-to-blood efflux transport				
P-gp/MDR1	Cyclosporin A and others	+	TR-iBRB	5,42,98
ABCG2	Mitoxantrone and others	+	TR-iBRB	104
MRP4	E17βG and organic anions	Ex vivo	ND	103 (This review)
Oatp1a4	E17βG and organic anions	+	ND	71,89,91
Oatp1c1	Organic anions	Ex vivo	ND	71
Oat3	PAH, PCG and 6-MP	+	ND	94

+ Immunohistochemical study; *ND* not determined; *RUI* retinal uptake index method; *TR-iBRB* a conditionally immortalized rat retinal capillary endothelial cell line; *DHA* dehydroascorbic acid; *GABA* γ-aminobutyric acid; *MTF* methyltetrahydrofolate; *E17βG* estradiol 17-β glucuronide; *PAH p*-aminohippuric acid; *PCG* benzylpenicillin; *6-MP* 6-mercaptopurine

retina and TR-iBRB cells and immunoelectron microscopy investigations revealed localization of CRT immunoreactivity in both the luminal and abluminal membranes of the inner BRB (26). CRT expressed in the luminal membrane would mediate creatine supply to the retina. CRT in the abluminal membrane may be involved in the uptake of metabolites of creatine since we recently demonstrated that CRT in the brush-border membrane of the choloid plexus is responsible for the uptake of creatinine and ganiginoacetate, which are the end metabolite and precursor of creatine, respectively, from the cerebrospinal fluid (59,60). Thus, the processes of creatine transport and CRT expression at the inner BRB are important for understanding the mechanism governing the supply of creatine to the neural retina and could help in the design of improved treatments for GA.

The retina produces more L-lactic acid aerobically than any other tissue (61). Moreover, L-lactic acid appears to be required as an energy source, in addition to D-glucose, in photoreceptors (62). Alm and Törnquist were the first to use the RUI method to show that L-lactic acid transport across the rat BRB exhibits saturability, pH-dependence, and is inhibited by pyruvate and 3-hydroxybutyrate (63). In addition to this functional evidence, Gerhart *et al.* used immunoelectron microscopy to provide morphological evidence that MCT1 (SLC16A1) is localized in both the luminal and abluminal membranes of the inner BRB and in the brush-border membrane of the outer BRB (64). TR-iBRB cells express MCT1 mRNA 33-fold more intensely than MCT2 (SLC16A7) mRNA (65). In a study of the transport characteristics of L-lactic acid at the inner BRB, [^{14}C]L-lactic acid uptake by TR-iBRB cells was shown to be a temperature-, H^+-, and concentration-dependent process with a Km of 1.7 mM L-lactic acid. L-Lactic acid uptake was inhibited by a protonophore, MCT inhibitors, and a number of other monocarboxylates and monocarboxylic drugs. Salicylic and valproic acids competitively inhibited this process with an inhibition constant (IC_{50}) of 4.7 mM and 5.4 mM, respectively (65). Although monocarboxylic drugs exhibit competitive inhibition for MCT1 transport, the choice of a carrier for drug delivery to the retina needs to be considered very carefully due to substrate specificity.

Folates play an essential role as cofactors for one-carbon metabolism in cells and, consequently, they are required for the *de novo* synthesis of purines, pyrimidines, and some amino acids, as well as for the conversion of homocysteine to methionine (66). Folate deficiency in the retina has been associated with increased risk of nutritional amblyopia and methanol-induced retinal toxicity (67,68). Much of the folate in the plasma of most mammals is in the reduced form, methyltetrahydrofolate (MTF), and its concentration is 5–50 nM. The concentration of total folate is about 0.7 nmol/g retina (equivalent to 700 nM) in the rat and about 0.1 nmol/g retina (equivalent to 100 nM) in humans (69). The [^{3}H]MTF uptake by TR-iBRB cells is Na^+- and Cl^--independent and concentration-dependent with a Km of 5.1 μM (70). This process is inhibited by reduced folate carrier 1 (RFC1/SLC19A1) substrates, such as methotrexate and formyltetrahydrofolate, in a concentration-dependent manner with an IC_{50} of 8.7 and 2.8 μM, respectively, suggesting that RFC1 mediates MTF uptake in TR-iBRB cells. RFC1 mRNA, compared with other folate transporters, such as proton-coupled folate transporter (RCFT/SLC46A1), is predominantly expressed in TR-iBRB cells and rat retinal endothelial cells freshly isolated using magnetic beads coated with rat-CD31 (platelet-endothelial cell adhesion molecule-1) antibody (which is generated for a marker and is exclusively and extensively expressed on the membrane of endothelial cells) (70,71). Since the Km of MTF uptake by TR-iBRB cells was found to be 5.1 μM and the plasma concentration of MTF is 5–50 nM, the role of RFC1 may be important in accurately assessing the efficacy of methotrexate in the retina in general.

Törnquist and Alm used the RUI method to demonstrate [^{3}H]L-arginine uptake from the circulating blood to the retina across the BRB and its inhibition by an excess of L-arginine, L-ornithine, and L-lysine, suggesting that the BRB supports L-arginine transport systems (72). In support of these *in vivo* experiments, RT-PCR analysis has been used to show that TR-iBRB cells and isolated rat retinal vascular endothelial cells express system y^+/CAT1 (SLC7A2) mRNA.

RECEPTOR-MEDIATED INFLUX TRANSPORT

SR-BI

Vitamin E is a family of essential micronutrients composed of lipid-soluble tocopherols and tocotrienols that have potent antioxidant activity (73,74). Among the vitamin E family, α-tocopherol is the major constituent found in mammalian tissues and has the highest biological activity. α-Tocopherol has been thought to be transported to the retina from the circulating blood by passive diffusion because it is a hydrophobic compound. However, α-tocopherol is exclusively associated with lipoproteins, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL), in the circulating blood. HDL is a more effective carrier of α-tocopherol than LDL based on a quantitative analysis (75). HDL-associated [^{14}C]α-tocopherol ([^{14}C]α-tocopherol-HDL) uptake by TR-iBRB cells exhibited a time-dependent increase and a temperature-dependence. The uptake of [^{14}C]α-tocopherol-HDL was inhibited by more than 50% in the presence of block lipid transport-1, a specific inhibitor of the scavenger receptor class B, type I (SR-BI)-mediated lipid transfer between HDL and cells (76), at concentrations of 0.1 to 10 μM (77). The expression of SR-BI protein was detected in TR-iBRB cells and immunostaining of SR-BI was observed along the rat retinal capillaries. The inhibition of SR-BI protein expression by SR-BI siRNA resulted in a 36% reduction in [^{14}C]α-tocopherol-HDL uptake (77). These results strongly suggest that SR-BI at the inner BRB is responsible for α-tocopherol uptake from the circulating blood.

Insulin and Transferrin Receptors

Insulin and transferrin receptors are expressed in the eye including the inner BRB (78,79). Exogenous genes are widely expressed in the mouse retina after intravenous injection of the monoclonal antibody to mouse transferrin receptor-pegylated immunoliposomes (PIL) encapsulated plasmid DNA (80). Using a monoclonal antibody to the human insulin receptor, PIL encapsulated plasmid DNA with an

opsin promoter is targeted across the BRB and into ocular cells. The β-galactosidase gene is expressed throughout the entire primate retina following the delivery of the gene to the eye via a trans-vascular route (81). Insulin and transferrin receptors are also expressed in the BBB (82). Although it is difficult to achieve preferential delivery to the retina, PIL non-viral gene transfer technology using an organ specific promoter like opsin makes gene delivery to the retina possible.

Integrins $\alpha_v\beta_3$ AND $\alpha_v\beta_5$

Integrins are a family of multifunctional cell-adhesion molecules composed of noncovalently associated α and β chains. They are transmembrane receptors that bind extracellular matrix components, such as vitronectin, fibronectin, laminin, collagen, fibrinogen, and thrombospondin. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ are involved in angiogenesis (83) and expressed in actively proliferating endothelial cells in human diabetic retinopathy, but not in normal tissues (84). Antagonists of integrins $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ are mostly effective in treating individuals with blinding eye disease associated with angiogenesis (85,86). Integrins $\alpha_v\beta_3$ and/or $\alpha_v\beta_5$ are targets of treatment for angiogenesis in diabetic retinopathy.

EFFLUX TRANSPORTERS AT THE INNER BLOOD-RETINAL BARRIER

Relapse of childhood acute lymphoblastic leukemia (ALL) involving the eye is a rare but challenging problem. One possible reason may involve the restricted distribution of 6-mercaptopurine (6-MP), which is frequently used in patients with ALL, in the eye (87). The distribution of β-lactam antibiotics in the vitreous humor/retina after systemic administration is restricted, resulting in reduced neurotoxicity and difficulties in treating bacterial endophthalmitis. In contrast, β-lactam antibiotics, such as carbenicillin and cefazolin, are rapidly eliminated from the vitreous humor compared with gentamicin and can be inhibited by probenecid (88). These pieces of evidence suggest that the inner BRB plays a role in acting not only as a structural barrier to regulate the passive diffusion of hydrophilic drugs but also as an efflux pump involved in the retina-to-blood efflux transport of xenobiotics and anionic drugs. We used microdialysis to carry out an *in vivo* evaluation of vitreous/retina-to-blood efflux transport in rats and to determine the efflux transport of organic anions across the BRB (89).

OATP1a4

[^{3}H]Estradiol 17-β glucuronide (E17βG) and [^{14}C]D-mannitol, which are used as a model compound for amphipathic organic anions and a bulk flow marker, respectively, were injected into the vitreous humor of the rat eye, and a microdialysis probe was placed in the vitreous humor (89). [^{3}H]E17βG and [^{14}C]D-mannitol were bi-exponentially eliminated from the vitreous humor after vitreous bolus injection. The elimination rate constant of [^{3}H]E17βG during the terminal phase was 1.9-fold greater than that of [^{14}C]D-mannitol, and it was significantly inhibited by organic anions including digoxin, a specific substrate of organic anion transporting polypeptide (Oatp) 1a4 (Slco1a4/Oatp2) (90). Gao *et al*. have provided immunohistochemical evidence that Oatp1a4 is present at the rat inner and outer BRB (91). Moreover, Oatp1a4 and 1c1 (Slco1c1/Oatp14) mRNA are predominantly expressed in isolated rat retinal endothelial cells (Fig. 2B) (71). Oatp1c1 has been identified as a BBB-specific anion transporter which has E17βG as a substrate (92,93). However, Oatp1c1 does not have a high affinity for digoxin (IC_{50} >0.5 mM) (93). Consequently, Oatp1a4 is suggested to be involved in the efflux transport of E17βG at the inner BRB.

OAT3

[^{3}H]*p*-Aminohippuric acid (PAH), [^{3}H]benzylpenicillin (PCG), [^{14}C]6-MP, and [^{14}C] or [^{3}H]D-mannitol are bi-exponentially eliminated from the vitreous humor after vitreous bolus injection (94). The elimination rate constant of [^{3}H]PAH, [^{3}H]PCG, and [^{14}C]6-MP during the terminal phase was about 2-fold greater than that of D-mannitol. This efflux transport was reduced in the retina in the presence of probenecid, PAH, and PCG, relatively specific substrates of organic anion transporter (Oat) 3 (SLC22A8) (95), whereas it was not inhibited by digoxin (94). We have used immunohistochemical staining in the inner BRB to show that Oat3 is co-localized with GLUT1, but not P-glycoprotein (P-gp/MDR1/ABCB1), suggesting that Oat3 is possibly located in the abluminal membrane of the inner BRB (94). GLUT1 is expressed in both the luminal and abluminal membranes of the inner BRB, and P-gp is localized in the luminal membrane of the inner BRB (5,14,15). Oat3 is most probably expressed in the abluminal membrane of the inner BRB and plays an important role in the efflux transport of PAH, PCG, and 6-MP from the vitreous humor/retina to the blood across the inner BRB.

ATP-Binding Cassette (ABC) Transporters

ATP-binding cassette (ABC) transporters are mainly present in the luminal membrane of retinal capillary endothelial cells and prevent distribution of drugs to the retina by exporting them from endothelial cells to the circulating blood. P-gp, which is an ATP-dependent 170 kDa membrane glycoprotein, exhibits a protective role by restricting the entry of a wide variety of chemotherapeutic agents and hydrophobic compounds into tumor cells as well as normal tissues (96). P-gp is present in the luminal membrane of the inner BRB (5), and the expression of mdr1a mRNA is predominantly exhibited in rat retinal endothelial cells. (Fig. 2C) (71). Moreover, no cyclosporin A was detected in the intraocular tissues of cyclosporin A-treated rabbits, although the blood level of cyclosporin A was within the therapeutic window (97). TR-iBRB cells express P-gp (42,98), and rhodamine 123 accumulation in TR-iBRB cells is enhanced in the presence of inhibitors of P-gp (98).

The ABC transporter superfamily consists of subfamilies A to G and 48 subtypes have been identified in humans (99). ABC transporter A (ABCA), B (ABCB), C (ABCC), and G (ABCG) play a role in transporting endobiotics and xenobiotics in the plasma membrane. ABCA1 and ABCG1 transport sterols (100). In addition to P-gp (ABCB1), the

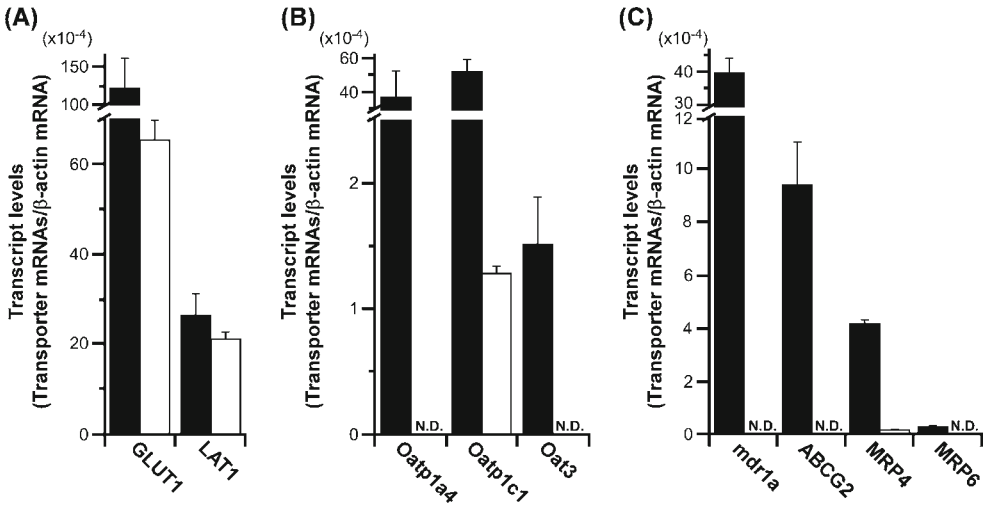

Fig. 2. Transcript levels of transporters in isolated rat retinal endothelial cells. The transcript level of influx transporters of nutrients (**A**), organic anion transporters (**B**), and ABC transporters (**C**) in rat retinal endothelial cells isolated using magnetic beads coated with anti-rat CD31 antibody was determined by quantitative real-time PCR analysis. Closed and open columns represent retinal endothelial cells and non-retinal endothelial cells fraction, respectively. Each column represents the mean ± S.E.M. of at least three different samples. N.D., not detected. GLUT1, Oatp1a4, Oatp1c1, and mdr1a: Data taken from the Journal of Neurochemistry, 941, Tomi and Hosoya, Application of magnetically isolated rat retinal vascular endothelial cells for the determination of transporter gene expression levels at the inner blood-retinal barrier. 1,244–1,248, 2004 (71) with permission from Blackwell Publishing. LAT1: Data taken from Investigative Ophthalmology & Visual Science, 46, Tomi *et al*., L-type amino acid transporter 1-mediated L-leucine transport at the inner blood-retinal barrier. 2,522–2,530, 2005 (23).

multidrug resistance-associated protein (MRP/ABCC) family and breast cancer resistance protein (BCRP/ABCG2) are known to be active efflux transporters of drugs (101). The MRP family plays a role in transporting anionic compounds, such as glucuronic acid conjugates and glutathione conjugates (101). The elimination of anionic drugs from the retina into the circulating blood consists of two steps, i.e., uptake across the abluminal membrane and subsequent excretion across the luminal membrane (102). In the abluminal membrane, Oatp1a4 and/or Oat3 appear to take up anionic drugs, followed by MRPs and ABCG2 in the luminal membrane.

Although the protein expression and localization of MRP and ABCA families are not yet fully understood, we recently demonstrated that ABCA3, ABCA9, MRP3 (ABCC3), MRP4 (ABCC4), and MRP6 (ABCC6) mRNA are highly expressed in isolated mouse retinal endothelial cells (103). We also quantified the gene expression levels of MRP4, MRP6, and ABCG2 in isolated rat retinal endothelial cells in this review (Fig. 2C). MRP4, MRP6, and ABCG2 mRNA are predominantly expressed in rat retinal endothelial cells compared with non-retinal endothelial cells.

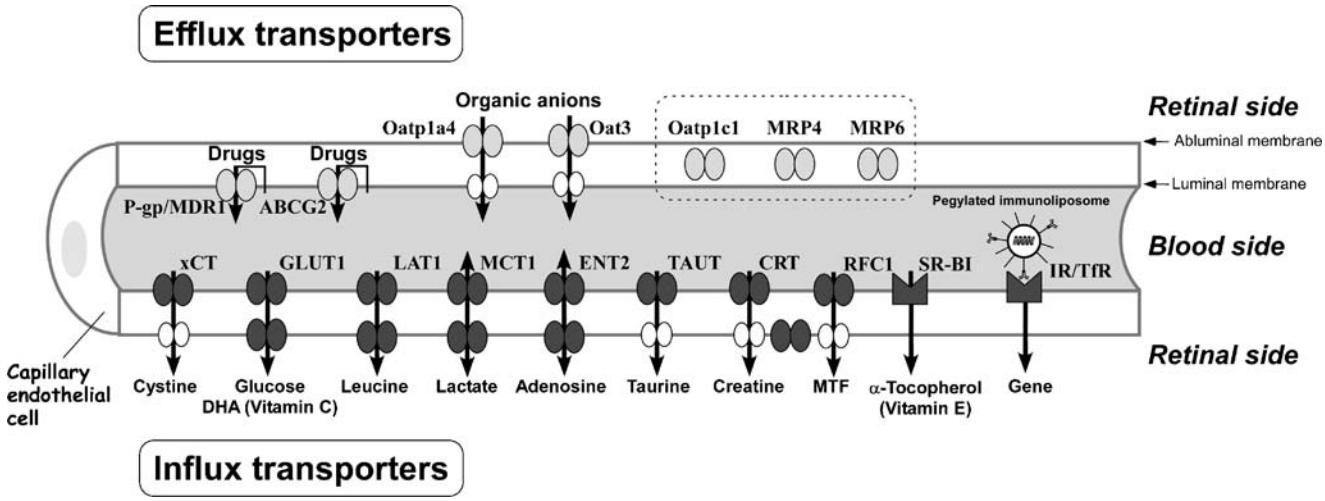

Fig. 3. Hypothetical localization and physiological function of the inner blood-retinal barrier transporters. Inner blood-retinal barrier transporters play an essential role in supplying nutrients to the retina and carrying out the efflux transport of endobiotics and xenobiotics. *DHA* dehydroascorbic acid; *MTF* methyltetrahydrofolate; *IR* insulin receptor; *TfR* transferrin receptor.

ABCG2 is reported to be expressed in the luminal membrane of the inner BRB (104). 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 retina is subject to high levels of cumulative irradiation and, therefore, vulnerable to light-induced damage caused by a variety of phototoxic compounds including porphyrins (105). TR-iBRB cells express ABCG2 protein, and Ko143, an ABCG2 inhibitor, inhibits the excretion of pheophorbide a from TR-iBRB cells (104). Taking these findings into consideration, P-gp, MRPs and ABCG2 are most likely expressed in the luminal membrane of the inner BRB and could act by restricting the distribution of endobiotics and xenobiotics including drugs in the retina. However, the contribution of each ABC transporter subtype to the inner BRB efflux transport is an important issue that remains to be resolved.

STRATEGIES FOR DRUG DELIVERY TO THE RETINA USING AN ACTIVE TRANSPORT SYSTEM

Carrier-mediated transport of drugs is of growing interest to many pharmaceutical scientists. Many transporters have been identified, and their substrate specificity has been characterized. Transporters play an important role in absorbing and distributing nutrients as well as their corresponding mimetic drugs. The transporter-mediated drug transport present at the inner BRB offers great advantages over passive diffusion, as the retinal endothelium acts as a tight-barrier. It is likely that paracellular transport across the inner BRB may be more restricted if we consider systemic administration. One approach is to increase the blood-to-retina influx transport of the drug and the other is to reduce the retina-to-blood efflux transport or total body clearance. Although making drugs more hydrophobic may lead to an increased inner BRB permeability, this benefit may be offset by an increase in the total body clearance and binding to plasma proteins. Therefore, it is important to design and select optimal drug candidates by taking into account the fact that drugs should be recognized by influx transporters and also that efflux transporters at the inner BRB should be avoided (Fig. 1). To screen compounds that are recognized by influx transporters, we have investigated whether amino acid mimetic-drugs are recognized by LAT1 as mentioned above (43). This approach is one strategy to increase influx transport across the inner BRB. However, LAT1 and other transporters are also expressed at the BBB (106,107) and drug distribution to the brain and side-effects on the central nervous system also need to be considered.

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 changing the drug distribution in peripheral tissues and the brain since efflux transporters are also expressed in peripheral tissues and the BBB (7,107).

CONCLUSIONS

Currently available experimental data suggest that the inner BRB is equipped with a variety of membrane transporters for influx transport of nutrients and efflux transport of endobiotics and xenobiotics (Fig. 3). LAT1 at the inner BRB appears to be involved in the influx transport of amino acid mimetic-drugs while Oatp1a4, Oat3, MRPs, and ABCG2 at the inner BRB appear to be involved in the efflux transport of anionic drugs. Information about influx and efflux transport will help in the design and selection of optimal drug candidates by taking into account the fact that drugs should be recognized by influx transporters and should also avoid efflux transporters. However, the relationship between the drug structure for transporter recognition and the pharmacophore model requires future investigation. As more information becomes available regarding inner BRB transporters, we may be able to design simpler and more effective routes for drug delivery to the retina and, consequently, improve the treatment of retinal diseases.

ACKNOWLEDGEMENT

This work was supported, in part, by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS). The authors thank Dr. M. Tomi for valuable suggestions, and S. Akanuma and T. Sugita for technical assistance.

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