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Blood-brain barrier transport machineries and targeted therapy of brain diseases

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Article Info

Abstract

Introduction: Desired clinical outcome of pharmacotherapy of brain diseases largely depends upon the safe drug delivery into the brain parenchyma. However, due to the robust blockade function of the blood-brain barrier (BBB), drug transport into the brain is selectively controlled by the BBB formed by brain capillary endothelial cells and supported by astrocytes and pericytes.

Methods: In the current study, we have reviewed the most recent literature on the subject to provide an insight upon the role and impacts of BBB on brain drug delivery and targeting.

Results: All drugs, either small molecules or macromolecules, designated to treat brain diseases must adequately cross the BBB to provide their therapeutic properties on biological targets within the central nervous system (CNS). However, most of these pharmaceuticals do not sufficiently penetrate into CNS, failing to meet the intended therapeutic outcomes. Most lipophilic drugs capable of penetrating BBB are prone to the efflux functionality of BBB. In contrast, all hydrophilic drugs are facing severe infiltration blockage imposed by the tight cellular junctions of the BBB. Hence, a number of strategies have been devised to improve the efficiency of brain drug delivery and targeted therapy of CNS disorders using multimodal nanosystems (NSs).

Conclusions: In order to improve the therapeutic outcomes of CNS drug transfer and targeted delivery, the discriminatory permeability of BBB needs to be taken under control. The carrier-mediated transport machineries of brain capillary endothelial cells (BCECs) can be exploited for the discovery, development and delivery of small molecules into the brain. Further, the receptor-mediated transport systems can be recruited for the delivery of macromolecular biologics and multimodal NSs into the brain.
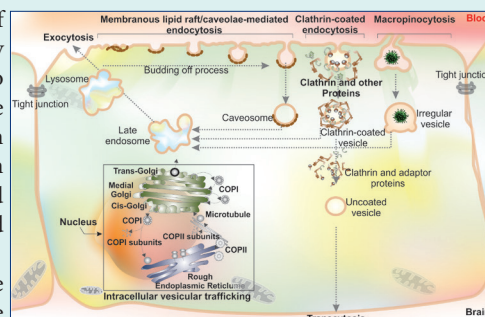
Introduction

Microcirculation of nutrients and drugs into the brain is selectively controlled by a unique biological barrier called blood-brain barrier (BBB). Such biological barrier is formed by the brain capillary endothelial cells (BCECs) whose maturation and functions is largely dependent upon intercellular interaction with brain astrocytes and pericytes.[1-3] Of the BBB cooperating cells, the astrocytes are members of the glial family, which are able to interact with neurons and other brain cells (e.g., microglia and endothelial cells) directly and/or indirectly through the exchange of soluble materials. At the BBB, the astrocytic foot process covers 99% of the abluminal surface of the capillary basement membrane, while the brain capillary pericytes share the basement membrane with BCECs (Fig. 1). The BCECs form a co-operating complex with astrocytes and pericytes (the "Neurovascular Unit") to: (a) maintain brain homeostasis, (b) selectively control the delivery of nutrients and blood-borne solutes to the brain parenchyma.[1] In addition to other modulation roles of astrocytes and pericytes on BCECs (e.g., restrictive tight junction (TJ) regulation and differentiation), they are also involved in modulating the functional expression of transport machineries required for the selective inward/outward transportation of nutrients and drugs.[4] It is now well-documented that the capability of a

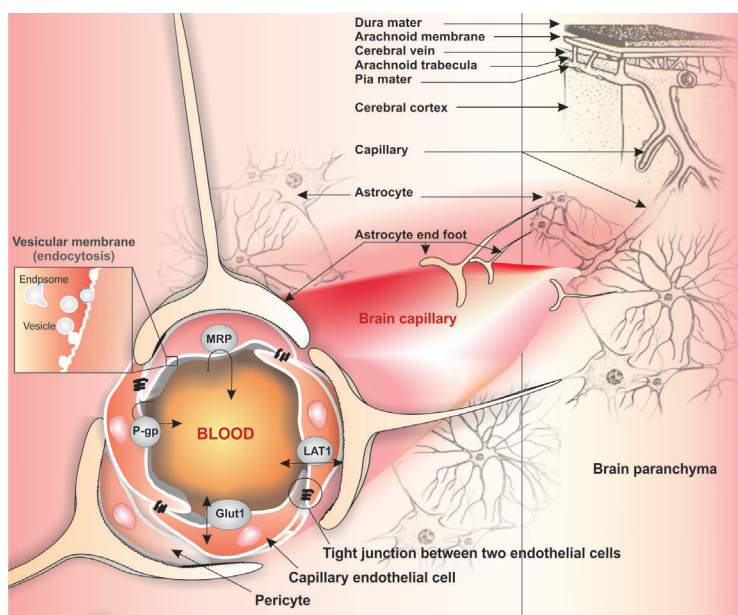*Corresponding author: Yadollah Omidi, Email: yomidi@tbzmed.ac.ir

Fig. 1. Schematic representation of the blood-brain barrier, astrocytes and pericytes. Image was adapted with permission from our previously published work.[1] Note: Not drawn to scale.

particular substance to cross the BBB and enter the central nervous system (CNS) depends on both the biological features (e.g., expression of transporters and enzymes) and the physicochemical properties of the compound (e.g., molecular weight, lipophilicity, pK_a, and hydrogen bonding capacity). The molecular trafficking through BBB can generally be classified into three categories, including: (a) passive diffusion that is largely dependent upon the lipophilicity of the molecule, (b) transport via one of a large number of solute carriers (SLC superfamily) such as those for glucose, organic anions, amino acids, and (c) vesicular trafficking system (VTS) through endocytosis and transcytosis.[5] Of these, the functional expression of carrier-mediated transporters (CMTs), which mediate both influx and/or efflux machineries, can selectively control transport of circulating small molecules between blood and brain. Small molecule (<500 Da) penetration into the brain parenchyma is dominated by passive transcellular diffusion with potential to be subject to efflux by members of the ABC (ATP-dependent binding cassette -ABC) superfamily such as P-glycoprotein (P-gp) (ABCB1) or BCRP (ABCG2) or a member(s) of the multi-drug resistance protein (MRP) family such as MRP1.[6,7]

In contrast polar hydrophilic small molecules (e.g., amino acids and carbohydrates) exploit the facilitative or passive SLC transport systems, while macromolecular biomolecules such as insulin, transferrin (Tf) and targeted nanoscale drug delivery systems (DDSs) use VTS pathways potentially involving a receptor-mediated transporter (RMT).[8,9]

In addition, it extracellular vesicles (exosomes and ectosomes) are engaged in macromolecular trafficking and cellular communications and can be used for targeted therapy of diseases such as CNS disorders.[10-14]

In this current review article, we provide an overview on the biological features of BBB relevant to brain drug delivery and targeting by highlighting the potential therapeutic importance of BBB transport machinery.

Experimental models for BBB

In order to evaluate the specific functions of BBB, *in vivo* and *in vitro* as well as *in silico* approaches have been employed by a number of research groups. Although the *in vivo* investigations provides much more reliable and robust physiological context measures of BBB functions, conducting animal based studies may encounter with some pivotal limitations (e.g., ethical issues of animal uses, cost and time restrictions, need for genetically modified animal models). Therefore, *in vitro* cell systems can serve as a complementray model to reduce and maybe refine later in-vivo investigations.[15] Cell-based models have capacity to provide relatively high trans-endothelial electrical resistance (TEER) values (i.e., about 1000 $\Omega.cm^2$), which offer some discrimination between transcellular and paracellular routes of permeation. We have previously examined several cell culture systems and found that the isolated primary cultures of brain capillary endothelial cells from porcine and bovine offer reliable outcomes.[1,4,16-19] Indeed the ability to use primary cell models or at the very least early low passage cells appears particularly important in BBB investigations. Clearly for human cell work much potential may arise through developments with induced pluripotent stem cells (iPSC).[20] Beyond permeation per se, cell models offer the potential for mechanistic transporter investigations.[18,21]

Brain drug delivery

A large number of studies on brain drug delivery and targeting, using *in vitro* cell-based BBB models[15-19] as well as animal models, have sought to address how the brain

capillary endothelium can be safely and efficiently crossed by experimental therapeutics and DDS. As shown in Fig. 2, the blood-borne substances, nutrients and drugs can enter into the CNS through one or more pathways each of which has been explored as a mean to overcome the BBB restrictiveness. The penetration of drugs into the brain may occur by following routs:

(a) paracellular transportation that is a pathway primarily for small hydrophilic substances. In this regard, there exist some investigations addressing transient relaxing of TJ regulation by means of osmotic22 or biologically active agents (e.g., bradykinin, cereport and histamine),[23-25]

(b) transcellular passive diffusion that is a primary route for small lipophilic substances, which could be optimized by altering drug physicochemical properties,

(c) SLC-mediated transport exploiting the diverse members of the SLC family of transporters preset within the BBB (e.g., various amino acids),[8,26]

(d) endocytosis through RMT (e.g., monoclonal antibodies (mAbs) specific to various receptors at the BBB like Tf receptor, targeted nanomedicines),[27-29] and

(e) adsorptive endocytosis or pinocytosis (e.g., albumin) a relatively low capacity route with non-receptor bound material shuttled to the lysosomes for degradation.

It should be noted that conjugation of a drug with a targeting peptide/protein or with an antibody (Ab)/aptamer (Ap) provides nanoconjugates that can specifically shuttle the conjugated drugs into the brain through RMTs.[30,31]

Besides, targeted nanosystems (NSs) such as polymeric/lipidic nanoparticles (NPs) decorated with homing entities also provide robust tools for crossing BBB that can be used to treat life-threatening diseases such as glioma.[32-34]

ABC transporters

The direct energy-dependent ABC active transporters, are primarily localized on the luminal membrane of brain microvasculatur cells. ABC transporters possess a common structure of transmembrane domains (TMDs) and cytosolic nucleotide binding domains (NBDs). The ABC superfamily encompasses about 48 distinct transporters categorized into 7 families (ABC-A,-B,- C,-D,-E,-F, and G). Of these, the most investigated transporter in the BBB is that coded by the *ABCB1* gene, ie. P-glycoprotein (P-gp or MDR1) (Fig. 3). As a phosphorylated glycoprotein (170 kDa), it plays extremely important role in tumor cells resulting in their resistance to anticancer drugs by effluxing xenobiotics and metabolites out of the cell. P-gp is expressed in both epithelial and endothelial cells that are involved in formation of biological barriers such as BBB. Table 1 represents ABC transporters function and clinical impacts. The functional expression of these transport machineries play key roles in the maintenance of brain homeostasis, in which traverse of blood-borne substances should be specifically regulated.

Impacts of solute carriers (SLCs)

The BBB carrier-mediated transporters belonging to the SLC superfamily are comprised of two functional groups that include the mediation of solute influx or efflux from the cell.

Influx CMTs

The SLC transporters involved in influx at the BBB may be divided into different groups, as follows:

(a) Hexose transport system for glucose and mannose such as glucose transporters (i.e., Glut-1 and Glut-3)[50,51]
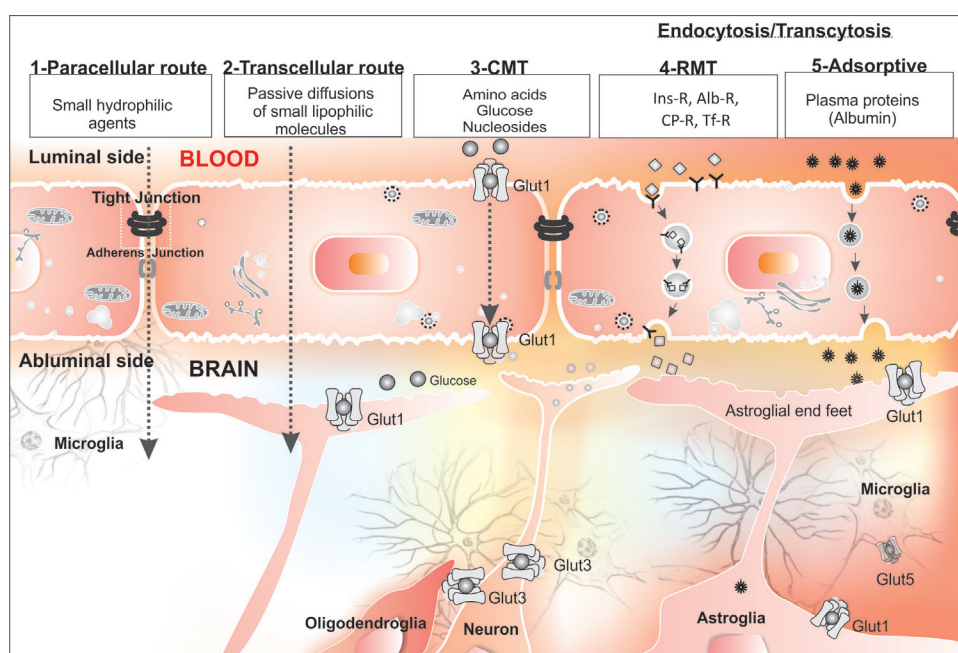
(b) Monocarboxylic acid transport (MCT) system such as

Fig. 2. Different mechanisms of drug trafficking from blood into the brain. Image was adapted with permission from our previously published work.[1] Note: Not drawn to scale.
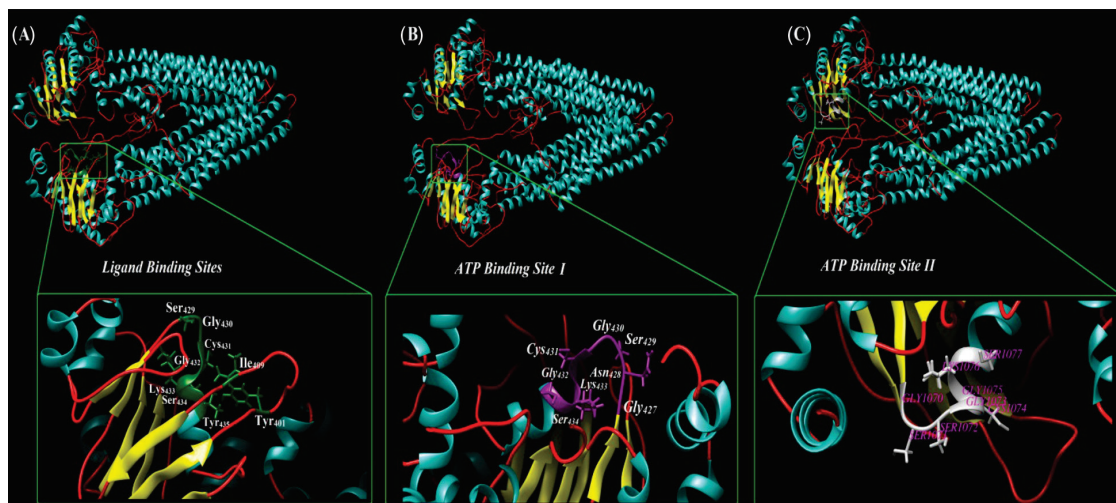
Fig. 3. Ribbon style representation of the modeled multidrug resistance protein 1 (MDR1). (A) The residues that are correlated ligand binding site are magnified. Panel B and C show the two ATP-specific binding sites in MDR1 structure. The UCSF Chimera *v*1.11 standalone software (freely available at: http://www.rbvi.ucsf.edu/chimera) and Visual Molecular Dynamics (VMD) (freely available at: https://www-s. ks.uiuc.edu/Research/vmd/) were used for visualization of MDR1.

MCT1 and MCT 8 for transportation of lactate, short-chain fatty acids, biotin, salicylic acid, valproic acid, phenylbutyrate and 3,5,3'-triiodo-L-thyronine[52-56]

(c) Neutral amino acids transporter (NAAT), system L, divided into large (LAT1) and small (LAT2) neutral amino acid transporter systems for transportation of neutral amino acids and some drugs such as L-dopa[57-59]

(d) Anionic amino acids transporters for transportation of aspartate and glutamate[60-62]

(e) Cationic amino acids transporter, system y⁺, for transportation of L-lysine, L-arginine, and L-ornithine[63,64]

(f) Beta amino acid transporter for transportation of β-alanine and taurine[65-67]

(g) Choline transport system for transportation of choline and thiamine through organic cation transporter (OCT) family[68-70]

(h) Nucleoside transport system[71]

(i) Peptide transporters, including oligopeptide transporters (e.g., PepT1, PepT2) and polypeptide transport system (e.g., Oatp2, OAT-K1, OATP)72,[73]

(j) Medium-chain fatty acid transporter.[74]

In the following, some brief discussion is provided on the clinical or biological importance of some of these SLCs at the BBB.

Hexose transporters
The functional expression of glucose transporters (GLUTs) was initially identified and characterized using D-glucose specific competitor cytochalasin B in the cerebral microvessels of rat and pig.[75] Later on, Pardridge and coworkers showed that the GLUT1 isoform is selectively expressed at the BBB by means of Western blotting, cytochalasin B binding, and *in situ* hybridization in bovine brain cortex. As a result, they concluded that the

GLUT1 gene is selectively expressed in the brain capillary endothelial cells, where 100% of the glucose transporter binding sites at the BBB can be attributed to the GLUT1 isoform.[51] Morgello *et al.* examined human and monkey brain sections recruiting immunoelectron microscopy. They showed GLUT1 expression in the gray matter and white matter endothelial cells, astrocyte foot processes surrounding gray matter blood vessels, and astrocytes adjacent to synaptic contacts. While the immunoblots of adult human frontal cortex were reported to have two forms of GLUT1 (45 and 52 kDa), the immunoblots of vessel-depleted frontal lobe showed the 45 kDa form solely in gray matter fractions. Such findings confirmed linkage to GLUT1 in both astrocyte and astrocyte-associated neuronal energy metabolism.[76] The modeled structure of GLUT1 is shown in Fig. 4. From a clinical viewpoint GLUT1 is highly upregulated at the BBB of patients with seizures,[77] and downregulated in Alzheimer's disease and diabetes.[78-79] Dysfunctional mutations in GLUT1 may lead to some deficiencies in the transportation of glucose in the related diseases.[80]

Monocarboxylic acid transport system
Monocarboxylate transporters (MCTs) consist of different isoforms. Of these, the MCT1, 2, 3 and 4 catalyze the proton-linked transport of monocarboxylates (e.g., L-lactate) across the plasma membrane,[82] the expression of MCT4 seems to be largely dependent upon the oxygen level of the cell as a hypoxia-dependent process.[83] Hypoxia may affect the expression of MCT1 and 2 and is mediated by the hypoxia inducible factor 1α (HIF-1α) that is overexpressed in tumor cells.[84] These transporters play pivotal metabolic roles in most tissues, including the brain and other tissues. The MCT8 and MCT10 are respectively involved in the transportation of thyroid hormones as well as some aromatic AAs through the BBB.[56,85,86] Inhibition of MCT1 by its specific inhibitors may provide a new

Table 1. ATP-binding cassette transporters' functions and clinical significances

Family	Members	Function	Clinical relevance
ABCA	Subgroup I: ABCA1, 2, 3, 4, 7, 12, and 13 Subgroup II: ABCA5, 6, 8, 9 and 10	Transportation of cholesterol and lipids	Mutation in ABCA1 causes Tangier's disease (hypoalphalipoproteinemia)[35]; Mutation in ABCA3 are associated to cataract-microcornea syndrome[36] Mutation in ABCA4 causes autosomal-recessive disease Stargardt macular dystrophy (STGD)[37]; ABCA7 involves in Alzheimer's disease[38]; Mutation in ABCA5 cause excessive hair overgrowth[39]; Mutations in ABCA12 cause severe congenital skin disease harlequin ichthyosis[40]
ABCB	ABCB1 (multidrug resistance protein 1 (MDR1)/CD243, the so-called P-glycoprotein 1 (P-gp)	Export of divers xenobiotics and toxic metabolites	P-gp transports various substrates across the BBB
ABCC	ABCC also known as multidrug resistance proteins (MRPs) members are MRP1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13	Anions and conjugated metabolites (e.g., glucuronides, glutathione) efflux	Involved in ion transport, toxin secretion, and signal transduction at BBB (MRP1, 2, 4, 5, 6); Deficiency in functional expression of ABCC7 (CFTR protein) causes cystic fibrosis[41]; Overexpression of MRP2 at BBB contributes to drug resistance in epilepsy[42]; MRP4 involves in efflux of oseltamivir[43]
ABCD	ABCD1	Transportation of long chain fatty acids	Mutations in ABCD1 cause accumulation of very long chain fatty acids; ABCD1 dysfunction at BBB contributes to the increased trafficking of leucocytes across the BBB as seen in cerebral adrenouleukodystrophy[44]
ABCE	ABCE1	ABCE1 inhibits the action of ribonuclease L	Overexpression of ABCE1 promotes growth and inhibits apoptosis of tumor cells[45]
ABCF	ABCF1	Export of some drugs	ABCF1 associates with Graves' ophthalmopathy[46]; ABCF1 may play a role in mediating antidepressant response[47]
ABCG	ABCG1, 2, 3 and 4	Transportation of lipids, steroids, cholesterol and different drugs	Loss of ABCG1/ABCG4 functions in the CNS alters metabolic pathways and elicits behavior changes[48]; Functional expression of ABCG2 at BBB results in markedly high efflux of divers drug substances[49]

treatment horizon in cancer chemotherapy in particular for glioblastomas (GBM).[87,88] This type of malignancy with high cellular heterogeneity and conspicuous necrotic regions is largely associated with hypoxia, in which the functional expressions of MCT1 and GBM hypoxia marker carbonic anhydrase 9 (CAIX) play key roles. It seems that dysregulated pH in the tumor microenvironment (TME) may pose a significant role in metabolic path of such malignancies.[89,90]

Amino acid transporters

Several amino acid transporters function at the BBB to maintain brain homeostasis. Of these, the glycoprotein-associated amino acid transporters (gpaATs) play an important role in AAs homeostasis in brain parenchyma. These transporters are basically part of the amino acid/polyamine/choline (APC) superfamily that is considered as one of the largest families of transporters. Table 2 lists some of the key amino acid transporters. The function of neutral amino acid transporter(s) is of note due to their involvement in several crucial events in CNS including synthesis of the neurotransmitters and peptides/proteins. Based upon the binding affinity of a particular transporter to AAs, along with its dependency to sodium ion, amino acid transporters can be classified into different groups such as system L, y+L, x_c^- (consisting of xCT and 4F2hc), asc, and b$^{0,+}$. These transporters show some commonalities and differences in terms of binding affinity to different amino acid substrates. For example, alanine can be transported into the brain through systems L and/or asc, which is an overlapped function between these transporters. For instance, system L plays a key role upon traverse of the neutral AAs (NAAs) in and out of the brain, maintaining desired concentrations of NAAs and hence affecting the NAA-related disorders such as depression. System L is a member of the gpaATs family, and possesses putative 12-transmembrane segment topology and comprises of light (LAT) and heavy (4F2hc) chains and two types of LAT1/4F2hc and LAT2/4F2hc.[57] System L and other members of gpaATs including y+LAT1, y+LAT2 and xCT share somewhat commonalities in terms of amino acid identity (around 40%-89%).

Furthermore, some of these transporters act as obligatory exchangers in an intricate holistic manner and play key roles in the mental health and neurological diseases through maintaining required concentrations. For example, xCT/4F2hc functions as the exchanger of L-cystine and L-glutamic acid.[95] Of these amino acid transporters, system y+L functions as a Na+-dependent carrier for transportation of NAAs. Further, emergence of mutations in systems b$^{0,+}$ and y+L transporters can respectively lead to cystinuria and lysinuric protein
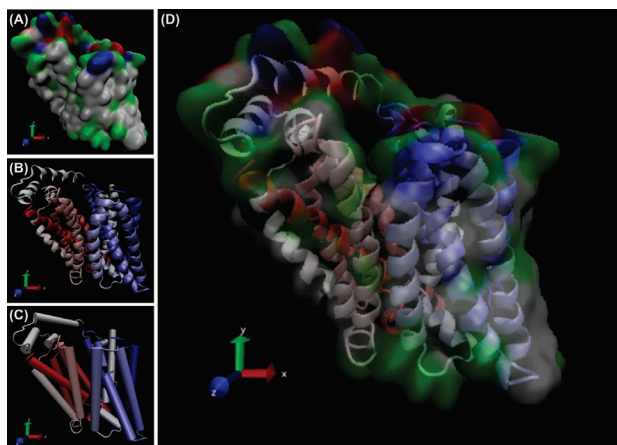
Fig. 4. The modeled structure of GLUT1 as an ABC inward transporter. Panels A, B and C represent different modes of the protein with 12 transmembrane helices. Panel D shows the superimposed image of the space filling structure (panel A) and the ribbon style model (panel B). The visual molecular dynamics (freely available at: https://www-s.ks.uiuc.edu/Research/vmd/) was used to visualize the GLUT1 in different modes.[81]

intolerance that are considered as inevitable hereditary diseases.[96] For example, two distinct genetic disorders, cystinura and Hartnup disease, are related to the sodium-dependent neutral and basic amino acid transport protein rBAT (SLC3A1) and neutral amino acid transporter B0 AT1 (SLC6A19).[97-101] In regards to the sodium-dependency of the ABC transporters, System L, System y+ and System Xc– are further reviewed in the following sections.

System L

The system L functions as a Na^+-independent bidirectional transporter that is responsible for both branched and aromatic neutral amino acids in almost all types of cells with different affinity to a range of large and small neutral amino acids.[57,58,102,103] As shown in Table 3, LAT1 is a ubiquitous transporter with a high affinity for LNAAs and with K_m values in the μM range, whereas LAT2 shows a lower affinity to LNAAs and higher affinity to SNAAs and possessing a K_m value in the mM range for NAAs.[58,102,104,105] Fig. 5 represents the membrane topology model of glycoprotein-associated amino acid transporters (*gpaATs*, i.e., SLC7A5-11) including system L (LAT1/4F2hc and LAT2/4F2hc heterodimers).[103] The heterodimeric complexation of 4F2hc with LAT1 or LAT2, through a covalent disulfide band, is in favor of functionality of the light chains and their co-expression as heterodimer complex - essential for transport functionality of the system. Table 2 represents AAs transporters whose associated heavy chain is the 4F2hc/rBAT.

In the absence of 4F2hc, the LAT1 is found in the intracellular compartments. Upon complexation with 4F2hc, it can reach to the plasma membrane, while the 4F2hc itself reaches the plasma membrane independently. The surface conformation of 4F2hc seems to be altered in the presence of LAT1, confining it to cell-cell adhesion sites. The 4F2hc comprises 529 amino acids (94 kDa,

glycosylated; 72 kDa unglycosylated). The light chain LAT1 is widely expressed in non-epithelial cells including brain, spleen, thymus, testis, skin, liver, placenta, skeletal muscle, and stomach.

In brain, LAT1 expression is found predominantly in capillary endothelial cells that form the BBB. In human, the LAT1 protein consists of 507 AAs with a molecular mass of 55 kDa, whose association with 4F2hc (LAT1/4F2hc heterodimeric complex) induces transportation of LNAAs into the brain.[58,106] Affinity of LAT1/4F2hc and LAT2/4F2hc transporters vary for different AAs. In addition to its role in neuronal cell proliferation (neurogenesis) in brain, system L shows affinity to thyroxin (T4) and triiodothyronine (T3). Table 2 represents the K_m values for some AAs in both subunits of the system L. Depending on the site of the biological membrane studied, luminal and abluminal transportation of AAs show different pattern. Since system L functions bi-directionally, the inward and outward traverses of AAs appear to differ one another, significantly. For example, the outward trafficking of L-phenylalanine seems to be greater than L-leucine whose inward transportation happens to be markedly high. A number of studies have shown that the subunits of system L (LAT1 and LAT2) act as an obligatory exchanger, so that the inward transportation of one AA may associate with the outward transportation of other AA.[107] Such exchange functionality, which may be indirectly related to ATP-dependent transporters activities, seems to provide the driving force for the Na^+-independent AA transport systems including system L. Nevertheless, the molecular mechanism(s) of this process is yet to be fully understood.

Of note, L-glutamine (Gln) is known as one of the most copious AAs in plasma and cerebrospinal fluid. It is a precursor for the CNS neurotransmitters such as gamma-aminobutyric acid (GABA). In an interesting study, Dolgodilina *et al.* analyzed the concentrations of Gln and also 13 other brain interstitial fluid AAs in mice by means of hippocampal microdialysis technique. Given that the interstitial fluid levels for all AAs including Gln were found to be approximately 5-10 times lower than in cerebrospinal fluid, they showed that the competitive inhibition of systems L transporter by 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) could significantly increase the concentration of Gln in interstitial fluid in comparison with inhibition of system A. They proposed an independent homeostatic regulation of AAs through system L transport machinery (LAT1/4F2hc) in the interstitial fluid in comparison with the cerebrospinal fluid.[113] Since LAT1 is an important transporter for drug delivery into the brain through CMT machinery, Ylikangas *et al.* developed a comparative molecular field analysis (CoMFA) model for the guided synthesis of LAT1-specific novel prodrugs which were examined using an *in situ* rat brain perfusion technique. These researchers concluded that L-tryptophan (Trp) can be used as the promoiety for the creation of novel LAT1-specific prodrugs with high-affinity.[114] Likewise, in a

Table 2. The glycoprotein-associated amino acid transporters at BBB

Transporter system	Light chain subunit	Heavy chain subunit	Tissue/ Localization	Function	Transport type		Pathology
					Influx	Efflux	
L	LAT1 (*SLC7A5*)	4F2/CD98 (*SLC3A2*)	Ubiquitous: ovary, placenta, brain>spleen, testis; activated lymphocytes; some tumor cells	L-type AAT 1; Na$^+$-independent, prefers L over A, accepts BCH, functions as an exchanger	L,H,I,F,Y,W, V,(M,Q)	L, others n.d.	n.d.
	LAT2 (*SLC7A8*)	4F2/CD98 (*SLC3A2*)	Kidney proximal tubule, small intestine>>ovary, placenta, brain	L-type AAT 2; higher affinity to small NAAs (e.g. A) than LAT1, functions as an exchanger; basolateral in transporting epithelia	F,Y,W>T,I,C,S,V,L,Q, A,H,N,M>(G)	F,I,L, others n.d.	n.d.
y$^+$ L	y+LAT1 (*SLC7A7*)	4F2/CD98 (*SLC3A2*)	Kidney, small intestine, leukocytes, lung, erythrocytes, placenta/ basolateral, brain	Na$^+$-dependent large NAAs/ dibasic AAs exchanger	Na$^+$-dependent: L,Q,M,H^0, (A,F); Na$^+$-independent: (R,K,H+)	R, no L, others n.d.	Lysinuric protein intolerance (y+LAT1)
	Y+LAT2 (*SLC7A6*)	4F2/CD98 (*SLC3A2*)	Brain (glia, neurons), small intestine, testis, parotis, heart, kidney, lung, liver/ basolateral	Na$^+$-dependent NAAs/dibasic AAs exchanger; glutamine/arginine exchanger	similar to y$^+$LAT1	n.d.	n.d.
X^-_c	xCT (*SLC7A11*)	4F2/CD98 (*SLC3A2*)	Macrophages, liver, kidney, brain, retinal pigment cells/basolateral	Glutamate/cystine exchanger	C-C>E	E	n.d.
asc	ascAT1 (*SLC7A10*)	4F2/CD98 (*SLC3A2*)	Brain, lung, placenta, small intestine, kidney/ basolateral	Na$^+$-independent exchanger of small NAAs, also D-serine, D-glycine	A,G	n.d.	n.d.
B$^{0,+}$	b0,1AT (*SLC7A9*)	rBAT (*SLC3A1*)	Kidney, small intestine, brain/ apical	Na$^+$-independent exchanger of neutral/ dibasic AAs; cystine, arginine, lysine, and ornithine reabsorption	C-C,R,K,>L,Y,A	L,R,A other n.d.	Cystinuria type I (rBAT) and non-type I (b0,1AT)

The gene name for each transport system is presented in the bracket. The capital single letter identity was used to present amino acids. C-C: L-cystine; H$^+$: the protonated L-histidine; H^0: the zwitterionic form of L-histidine. AAs: amino acids; AAT: amino acid transporter; NAAs: neutral amino acids; diAAs: diamino acids; n.d.: not determined. For detailed information, readers are referred to the following cited articles.[91-94]

previous study, four phenylalanine derivatives of valproic acid were designed and examined for their potential to utilize the LAT1-mediated transportation. In this study, a novel meta-substituted phenylalanine analogue promoiety with high affinity to LAT1 was introduced – a proof of application for the LAT1-mediated brain targeting.[115] In fact, despite ubiquitous expression of the LAT1 and LAT2 transporters by BCECs, only a few drugs (e.g., L-DOPA, alpha-methyldopa, melphalan, and gabapentin) have been developed as the substrates of LAT1 and LAT2. This could be due to possible ambiguousness in the pharmacokinetics of LAT1 and LAT2 transporters because of involvement of other amino acid transporters.[59,116]
Like any other AA transporters expressed by the BCECs, the impacts of system L transport machinery in healthy

and diseased brain are yet to be fully elucidated. In fact, CNS disorders such as schizophrenia, depression and Parkinson appear to be largely dependent upon the brain uptake of LNAAs such as Trp and L-tyrosine (Tyr) through LAT1 and homeostasis of serotonin. Therefore, any malfunctioning of system L (e.g., mutations or other genetic defects) can affect the homeostasis of LNNAs that are precursors of various neurotransmitters. What is more, it seems that LAT1 and LAT2 functions are complementary, in which LAT2 can mediate the outward transportation of various AAs such as LNAAs that are not competently transported through the unidirectional transporter located in the same membrane.
It appears that a complementary functioning transport systems such as y+LAT1 are involved in maintaining the
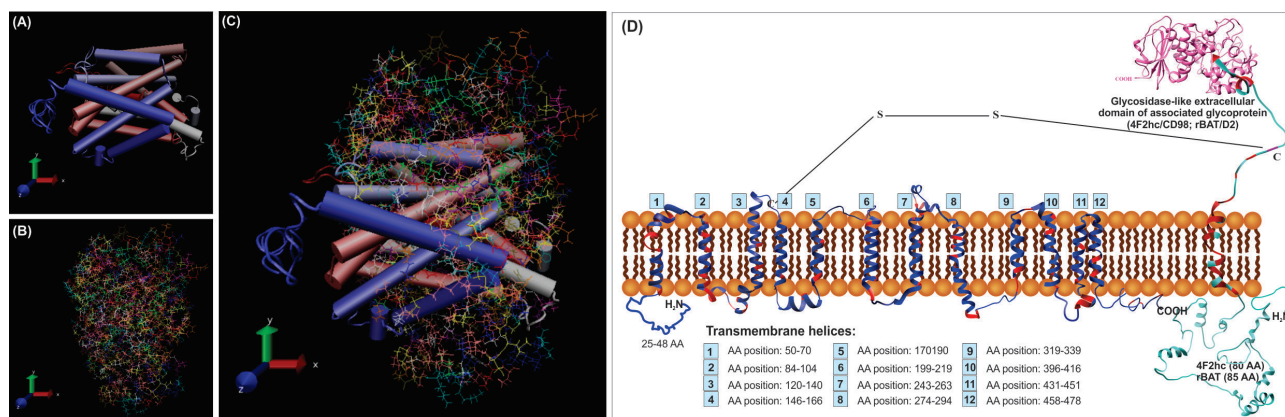
Fig. 5. Predicted membrane topology of the glycoprotein-associated amino acid transporters (*gpaATs*) in association with type II surface glycoprotein (4F2hc/CD98 or rBAT/ D2). Panels A, B and C represent 3D models of LAT1 (SLC7A5), 4F2hc (SLC3A2) and their interacted structures, respectively. Panel D shows the assumed interaction of *gpaATs* and *CD98/D2* with cell membrane. The conserved regions of gpaATs are drawn as *red color*. The amino acids (AAs) of the aligned sequences (human and mouse LAT1, LAT2 and y⁺LAT1; human y⁺LAT2; mouse xCT; human, rat, mouse and rabbit b⁰,⁺AT) show some commonalities and differences. There are some AAs that are not present in all aligned sequences. Numbers 1-12 show the predicted position of the putative transmembrane helices. The two-third predicted transmembrane segments could take an α-helical structure. For function of *gpaATs*, they need to be coupled with their related heavy chain (4F2hc/rBAT). As showed in panel D; gpaATs have an intramolecular covalent disulfide bridge (S–S) with their associated type II glycoprotein. Human rBAT and 4F2hc have six and three possible N-glycosylation sites, respectively. For detailed information, readers are referred to the following cited articles.[91] I-TASSER web-server has been utilized for homology modeling of this complex protein. Transmembrane sections were also identified by use of UniProt detailed annotations (http://www.uniprot.org/). The UCSF Chimera *v*1.11 standalone software (freely available at: http://www.rbvi.ucsf.edu/chimera) and Visual Molecular Dynamics (VMD) (freely available at: https://www-s.ks.uiuc.edu/Research/vmd/) were used for visualization of LAT1/4F2hc.[81] Note: Not drawn/modeled to scale.

CNS homeostasis from AA concentration viewpoints.[112] Further, on the ground of anomalous overexpression of the LAT1 on the membrane of different types of cancerous cells,[117-121] this transporter is considered as a potential cancer marker molecule for targeted therapy.[122-124] For example, qRT-PCR and Western blot analyses of the expression of three cancer-associated CMTs involved in the traverse of AAs (i.e., LAT1, ASCT2 and SN2) have revealed that the LAT1 was markedly upregulated in human ovarian cancer cells (SKOV3, IGROV1, A2780, and OVCAR3 cell lines as compared to the normal ovarian epithelial IOSE397 cells), whereas the functional expression of ASCT2 and SN2 transporters was not altered.[117] Therefore, the LAT1 has been considered as a target for the combinational therapy of cancer using antiproliferative agents such as aminopeptidase inhibitors. In 2015, Wu *et al.* developed a LAT1-mediated chemotherapeutic agent using aspartate-conjugated doxorubicin (Asp-DOX) with high affinity to LAT1. These researchers showed that the Asp-DOX could substantially inhibit the LAT1 overexpressing cancer cells. Such findings were further confirmed by pharmacokinetic data through significant accumulation of Asp-DOX within the tumor tissue in HepG2 and HCT116 tumor-bearing mice most likely through LAT1 uptake. Upon which, the Asp-DOX was proposed as a new treatment strategy for the LAT1 overexpressing cancer cells.[124] Owing to high expression of the LAT1 at the BBB, prodrugs can be developed to mimic the AAs that are substrates of LAT1. These prodrugs should ideally possess both the negatively charged alpha-carboxyl and the positively charged alpha-amino groups.[125]

System y+

Transportation of cationic amino acids (CAAs) is mediated through two families of proteins that are abundant in different cells/tissues. These families are (a) system y+ so-called CAA transporters (CAT), and (b) broad-scope amino acid transporters (BAT) consisting of systems b0,+, B0,+, and y+L. The functional expression and distribution of CATs and BATs by BCECs have not been fully addressed. Plasma membranes of bovine brain microvessels have been isolated and used to identify the CAAs transporters at BBB; as a result, the system y+ was found to be the only transporter present, mostly on the abluminal membrane. It showed voltage dependency and K_M values at µM range for AAs such as L-lysine, L-arginine, and L-ornithine. The system y+, in the presence of Na+, was inhibited by several essential neutral AAs with K_i values of 3-10 times greater than the plasma concentrations. However, some small nonessential AAs (i.e., serine, glutamine, alanine, and glycine) were able to inhibit the system y+ with K_i values similar to their plasma concentrations, implying that the system y+ might be involved in BBB traverse of these AAs. Further, the system y+ could be a key transporter in traverse of arginine that is necessary for the synthesis of nitric oxide (NO) in cerebral endothelial cells.[63] Taken all, it seems that this system is the only CAA transport machineries at BBB.[63,64] It should be also stated that y+LAT1 (SLC7A7) is highly expressed at the basolateral membrane of epithelial cells in the intestine and kidney. It is involved in transportation of dibasic AAs, and emergence of mutations in this transporter can lead to lysinuric protein intolerance (LPI). It is an inherited aminoaciduria that is a rare recessive disorder

Table 3. Affinity of system L transporters to different amino acids and drug/chemical substances

Amino acid	Affinity (K_M value)		Condition/model	Ref.
	LAT1/4F2hc	LAT2/4F2hc		
Thyroxin (T4)	7.9 µM	–	*Xenopus laevis* oocyte and presence of choline chloride	[108]
Triiodothyronine (T3)	0.8 µM	–	*Xenopus laevis* oocyte and presence of choline chloride	[108]
Reverse triiodothyronine (rT3)	12.5 µM	–	*Xenopus laevis* oocyte and presence of choline chloride	[108]
3,3'-diiodothyronine (T2)	7.9 µM	–	*Xenopus laevis* oocyte and presence of choline chloride	[108]
L-Phenylalanine	14.2 µM	45.0 µM	*Xenopus* oocyte	[109, 110]
	55.2 µM	–	T24 human bladder carcinoma cells	[104]
L-Isoleucine	25.1 µM	96.7 µM	*Xenopus* oocyte	[109, 110]
L-Methionine	20.2 µM	204.0 µM	*Xenopus* oocyte	[109, 110]
L-Valine	47.2 µM	124.0 µM	*Xenopus* oocyte	[109, 110]
L-Histidine	12.7 µM	181.0 µM	*Xenopus* oocyte	[109, 110]
L-Tyrosine	28.3 µM	35.9 µM	*Xenopus* oocyte	[109, 110]
	60.4 µM	–	T24 human bladder carcinoma cells	[104]
	16.4 µM	–	Human fibroblasts	[111]
L-Leucine	19.7 µM	151.0 µM	*Xenopus* oocyte	[112]
L-Tryptophan	21.4 µM	57.6 µM	*Xenopus* oocyte	[109, 110]
L-Asparagine	1.6 mM	80.7 µM	*Xenopus* oocyte	[109, 110]
L-Glutamine	2.2 mM	275.0 µM	*Xenopus* oocyte	[109, 110]
L-Threonine	–	68.6 µM	*Xenopus* oocyte	[110]
L-Alanine	10.0 mM	978.0 µM	*Xenopus* oocyte	[110]
L-Glycine	–	265.0 µM	*Xenopus* oocyte	[110]
L-Serine	–	116.0 µM	*Xenopus* oocyte	[110]
L-Dopa	34.2 µM	–	T24 human bladder carcinoma cells	[104]
3-O-Methyldopa	96.5 µM	–	T24 human bladder carcinoma cells	[104]
α-Methyltyrosine	153.0 µM	–	T24 human bladder carcinoma cells	[104]
α-Methyldopa	216.0 µM	–	T24 human bladder carcinoma cells	[104]
Gabapentin	191.0 µM	–	T24 human bladder carcinoma cells	[104]
Melphalan	73.5 µM	–	T24 human bladder carcinoma cells	[104]
BCH	156.0 µM	–	T24 human bladder carcinoma cells	[104]

For detailed information, readers are also referred to the cited articles. BCH: 2-Aminobicyclo-[2, 2,1]heptane-2-carboxylic acid.

with certain symptoms such as growth retardation, muscle hypotonia and hepatosplenomegaly. Technically, LPI patients are compound heterozygote with a missense mutation(s) in one allele and a frameshift mutation in the other.[126,127] Defective functionality of the y+LAT1 was reported to be influential on the functional expression of y+LAT2 (SLC7A6), perhaps through interference with its compensatory functions in LPI patients.[128] The pathological consequences of such mutations at the BBB are yet to be fully understood.

System Xc⁻
As an ABC transporter the system Xc⁻ (SLC7A11) possess 12 transmembrane helices and extracellular and cytoplasmic domains (Fig. 6). It is critical in AA exchanger function in various cell types such as BCECs.
Given the importance of glutathione (GSH) inside and outside of the CNS, several mechanisms are involved in its regulation, including Nrf2-ARE signaling pathway.[129] Further, the synthesis of GSH is attributed to some enzymes as well as CMTs such as system Xc⁻ and excitatory amino acid transporters (EAATs) such as EAAT1, 2 and 3.[130]

Fig. 7 represents the inward and outward trafficking of L-cysteine and L-glutamine by the brain capillary endothelial cells. At the BBB, the endothelial cells functionally express the EAATs on the abluminal membrane to shift the glutamate molecules from the extracellular fluid (ECF). This function controls the traverse of glutamate molecules in the CNS, resulting in selective maintenance of the low glutamate concentrations in the ECF within the CNS. As a result, the BBB remains almost impermeable to L-glutamate even at high concentrations. However, some diseases (e.g., diabetes[131]) can compromise the integrity of BBB.[132] It should be noted that the excessive amounts of glutamate can result in toxic impacts in the neurons, while the EAATs in cooperation with system Xc⁻ recycle the extra amount through efflux and production of GSH. Based upon the glutamate-glutamine cycle, the neurotransmitter glutamate is adequately maintained in the CNS, where the neurons are not able to synthesize the neurotransmitter as well as the γ-aminobutyric acid (GABA) from glucose. There is a glutamate-glutamine cycle between neurons and astrocytes, at which the liberated glutamate/GABA
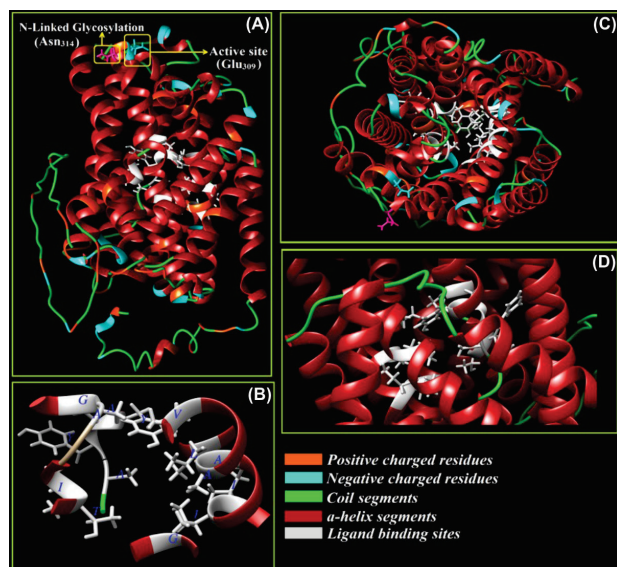
Fig. 6. Three-dimensional structure of ATP-binding cassette transporter, system Xc⁻. The Na⁺-independent system Xc⁻ functions as L-cysteine/L-glutamate antiporter. Panel A shows an active site (Glu309) and N-glycosylation residue (Asn314). Panels B, C and D show the ligand binding site residues (Thr56, Ile57, Ala60, Gly61, Ile133, Ile134, Ala137, Ala138, Val141, Tyr244, Ala245, Tyr246, Ala247, Gly334, and Lue392) from different prospects. The positive and negative charged amino acids, α-helix and coil regions of the model are also indicated. The structure is modeled through I-TASSER web server and based on threading and homology modeling approaches and is visualized by UCSF Chimera *v*1.11 software (our unpublished data).

molecules from neurons can be taken up by the astrocytes that are able to convert it to glutamine.[133] The latter biomolecules can be used by neurons as precursors for the synthesis of glutamate or GABA. However, in the case of ischemic brain damages, the EAATs function reversely resulting in the accumulation of glutamate molecules in the ECF and hence the blockage of neurotransmission. At the BBB, the system Xc⁻ pumps the glutamine molecules out of the ECF of CNS into the endothelial cells and then to the blood. Further, the BCECS are also able to hydrolyze glutamine, and use it for production of GSH. In such process, harmonically orchestrated functions of various AA transport machineries such as Na⁺-dependent systems N (astrocytes) and A (neurons) as well as facilitative glutamate transporters maintain such homeostasis.[133] The role of BBB and transportation of glutamate has comprehensively been discussed recently, readers are referred to the cited article.[132]

Nucleoside trafficking

Trafficking of nucleosides and related anticancer and antiviral nucleoside drugs across the BBB is mediated through the nucleoside transporters (NTs) located on the plasma membrane of the BCECs. These transport machineries play pivotal role in maintaining the homeostasis of the nucleosides within the CNS, in particular the level of adenosine, to keep it available to bind to receptors, by which a variety of physiological
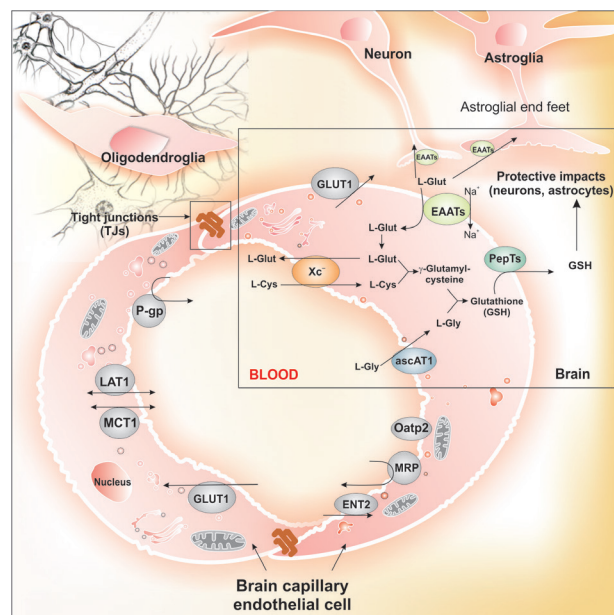

Fig. 7. Inward and outward trafficking of L-cysteine and L-glutamine by the brain capillary endothelial cells. System Xc– acts as the exchanger for amino acids such as L-cysteine and L-glutamine, and in close cooperation with excitatory amino acid transporters (EAATs) traverse of glutamate is controlled by its efflux from brain and production of glutathione. Glutamate as the most abundant excitatory neurotransmitter can be transported among brain capillary endothelial cells, neurons and astrocytes, where the endothelial cells of the blood-brain barrier play pivotal role in maintenance of glutamate in brain. Glutamate is stored in vesicles at the chemical synapses, where the impulses of nerve is able to activate the liberation of the glutamate molecules from the presynaptic cell. Its concentration in the extracellular space is controlled through Na+-dependent glutamate transporters (i.e., EAAT1, 2 or 3) found in neuronal and glial membranes. Any malfunctioning of EAATs may result in inadvertent accumulation of glutamate molecules causing inevitable excitotoxicity and interfering with the normal activity of neurons (our unpublished data). Note: Not drawn to scale.

processes are modulated.[134]

It should be stated that S-(4-Nitrobenzyl)-6-thioinosine (NBTI, NBMPR) riboside has been used for determination of the equilibrative nucleoside transport processes in mammalian cells, upon which these transporters are categorized into the NBMPR-sensitive (es) or NBMPR-insensitive (ei).[135] Previously, Chishty *et al*. aimed at examining the functional expression of CMT subtypes involved in the nucleoside transfer between blood and brain employing the RBE4 immortalized rat brain endothelial cells. Their findings revealed that the uptake of nucleosides by these cells was partially sodium dependent, which indicated that transportation occur in both equilibrative and concentrative manners. While these researchers reported that the uptake of adenosine via equilibrative transporter(s) are sensitive to NBMPR, they also showed the involvement of concentrative transporters in the uptake of adenosine which was significantly inhibited by subtype-specific inhibitors thymidine (*cit*) and formycin-B (*cif*), but not tubercidin (*cib*).[136] A number of studies have disclosed involvement of two major families

of membrane NTs, including (a) equilibrative nucleoside transporters (ENTs) and (b) concentrative nucleoside transporters (CNTs). ENTs transport nucleosides in a gradient-dependent fashion, while the CNTs import nucleosides against a gradient by coupling movement with sodium transport.[137] The ENT family consists of two subtype proteins in human cells (hENT1/SLC29A1 and hENT2/SLC29A2) that are involved in the transportation of purine and pyrimidine nucleosides down their concentration gradients. The human ENTs are either es (hENT1 with K_M values of 0.62 mM for adenosine) or ei (hENT2 with K_M values of 0.75 mM for adenosine and 0.2 mM for uridine).[135,138] Of the six functional subtypes of human CNTs, three of them (hCNT1/SLC28A1, hCNT2/SLC28A2 and hCNT3/SLC28A3) are considered as well-characterized Na$^+$–linked nucleoside cotransporters that are concentrative, insensitive to NBMPR and accept permeants.[139] These transporters act selectively, in which the hCNT1 (system *cit*) and the hCNT2 (system *cif*) transport respectively pyrimidine and purine nucleosides, while the hCNT3 (system *cib*) is involved in transportation of both pyrimidine and purine nucleoside.[140] The hCNT1 exhibits the transport properties of the NT system *cit* or N2 subtype (N2/*cit*) that is selective for pyrimidine nucleosides and adenosine. It is able to transport the antiviral pyrimidine nucleoside analogs 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxycytidine (ddC), while shows different affinity to nucleosides. The hCNT2 shows the transport characteristics of the NT system *cif* or N1 subtype (N1/*cif*) that is selective for purine nucleosides and uridine. The hCNT3 exhibits the transport features of the NT system *cib* or N3 subtype (N3/*cib*) that is selective for transportation of cytidine (K_M= 15.4 μM) thymidine (K_M= 21.2 μM), uridine (K_M= 21.6 μM), adenosine (K_M= 15.1 μM), guanosine (K_M= 43.0 μM) and inosine (K_M= 52.5 μM),[140,141] as well as gemcitabine, AZT, ribavirin and 3-deazauridine.[140]

Recently, Lepist *et al.* studied the transportation of tecadenoson, which is a selective A1 adenosine receptor agonist with similar structure to that of adenosine, into the CNS. Based on their findings, the binding affinities of the hENTs and hCNTs to tecadenoson largely varied showing the following pattern: hENT1 > hCNT1 > hCNT3 > hENT2 > hCNT2. They concluded that the hENT1 is the main transporter of the tecadenoson at the BBB.[142] In fact, the hENT1 is a ubiquitous transporter found in various human cells/tissues. The hENT2 is expressed in a variety of tissues such as the skeletal muscle where it plays a predominant role. Its functional expression in the brain microvasculature endothelial cells is yet to be fully addressed.

It should be highlighted that both pyrimidine and purine nucleoside analogs are currently used in clinic. Accordingly, various prodrugs can be developed to mimic these transporters capacities.[143,144] Of the pyrimidin analog drugs used in clinics, cytarabine (Cytosar-U®, Ara-C, DepoCyt®) is an analog of deoxycytidine (1-β-d-arabinofuranosylcytosine). It is used in the combination

chemotherapy against chronic myelogenous, leukemia, multiple myeloma, Hodgkin's lymphoma and non-Hodgkin's lymphomas. Gemcitabine (Gemzar®, Eli Lilly and Company, USA) has broad spectra applications in treatment of various malignancies such as pancreatic and bladder cancers. Capecitabine (Xeloda®, Roche, Switzerland) is a prodrug and analog of pyrimidin used orally for the treatment of metastatic gastric, colorectal, oesophageal and breast cancers. Of the purine nucleoside analogs, fludarabine (Fludara®) is used for the treatment of low-grade lymphomas. Cladribine (Leustatin®) is used for the treatment of hairy cell leukemia and for B-cell chronic lymphocytic leukemia.

Efflux CMTs

In addition to influx systems of CMTs at the BBB, brain capillary microvasculature embraces some key transport machineries that act as major hindrance against the entry of a number of different therapeutic drugs into the brain parenchyma – a function known as the efflux pumps of BBB. Of these, multidrug resistance 1 (MDR1/ABCB1)/P-gp as an ATP-binding cassette (ABC) drug transport protein seems to be the major efflux system at BBB that is predominantly found in the luminal sides of different endothelial and epithelial cells as well as polarized BCECs.[145,146] The putative transmembrane biostructure of human MDR1/P-gp is primarily found in the plasma membrane cell as 12 transmembrane segments that form a three-dimensional structure in the cell plasma membrane blocking the entry of various compounds. This polypeptide consists of two similar halves with six putative transmembrane segments and intracellular ATP-binding site (Fig. 3B, C). The hydrolysis of ATP biomolecules appears to maintain the active export of substrates by this transporter.

It has been shown that mouse and human P-gp transporters are able to actively export a number of compounds such as ivermectin, dexamethasone, digoxin, cyclosporin A and morphine across the polarized epithelial cells *in vitro*. In wild-type mice, the efflux of digoxin and cyclosporine was 20- to 50-fold higher than that of the mdr1a knockout mice.[147] Of the ABC transporters, in addition to MDR1/P-gp, the MRPs and cancer resistance protein (BCRP) expressed by BCECs can also pump out a large number of various compounds.[7] For example, it has recently been shown that both HIV-1 protease inhibitors (HPIs) and nucleoside reverse transcriptase inhibitors (NRTIs) are prone to be taken out by the efflux function of BBB through MRPs (e.g., MRP1 and MRP2).[148] Further, it has also been reported that the brain accumulation of sunitinib, as a multi-targeted tyrosine kinase inhibitor (TKI), is markedly restricted by both ABCB1 and ABCG2 efflux activities. Given that the full inhibition of these transporters can significantly increase the accumulation of sunitinib within the brain, combined oral administration of sunitinib with the P-gp inhibitor elacridar can be of great benefit in treatment of brain tumors.[149] While sunitinib is metabolized by CYP3A4,

perhaps rationalized combination of the CYP3A4 inhibitor (ritonavir) to such combination therapy could augment the impacts of pharmacotherapy, an extended exposure of BCECs to ritonavir was reported to impose a concentration-dependent increase in P-gp activity and expression. It has been shown that cyclosporin A (CysA) as a potent P-gp and MRP modulator, is able to moderate the concentration of methotrexate (MTX) in the rat brain. As a result, it is proposed as a useful tool to achieve an appropriate MTX concentration in brain in the case of brain tumors.[150] As another instance, it has been shown that antiepileptic drugs (AEDs) can only treat 30-40% of patients with epilepsy effectively. Such failure is deemed to be in large part due to the impacts of efflux functions of BCECs on outward transportation of AEDs which may be considered as a mechanism for resistance to AEDs in epileptic patients.[151] Of the AEDs, lacosamide (LCM) has been tested for its liability towards efflux functions in cell models by concentration equilibrium transport assays (CETAs). In addition, LCM can be taken up by MDR1 expressing cells through a passive diffusion, however it could be a substrate of P-gp in CETA.[151]

Peptide transporters

Peptides (mostly di-/tri-peptides) are normally crossed the BBB through peptide transporters found as an integral part of the plasma membrane proteins which also mediate the trafficking of peptide-mimetic drugs. It should be noted that the electrochemical Na$^+$ gradient, the so-called sodium-motive force (SMF) is the main driving force for the active/facilitated transportations of a variety of biomolecules such as AAs and carbohydrates in the most of living cells as well as the mammalian cells. The peptide transport process, nevertheless, seems to be an evolutionary shift in the coupling ion and favors H$^+$, through which a proton-motive force (PMF) energizes active traverse of peptides through cell membrane. Interestingly, various cellular transportation activities in mammalian cells are coupled with the Na$^+$/H$^+$ exchangers (NHEs), Na$^+$/K$^+$ pump and H$^+$ pump in terms of energy transduction.[152,153] Regulation of these cellular machineries orchestrate the solutes concentrations within the cells as well as the ECF, playing central roles in health and diseases. For example, in addition to the key role of NHE1 (SLC9A1) in dysregulation of pH in the tumor microenvironment (TME),[89] ischemia-reperfusion was shown to induce cardiac injuries that are mediated by NHE1. Its upregulation can modulate the activity of the plasmalemmal Na+/Ca2+ exchanger.[152] While NHE1 and NHE2 (SLC9A2) are the keystones in maintaining the volume and ionic compositions of brain interstitial fluid,[154] their roles in brain ischemic disease can be expected.[155]

Up until now, two peptide transporters, namely PepT1 (SLC15A1) and PepT2 (SLC15A2), have been discovered in human, which are known as proton-coupled transporters that influx oligopeptides of 2 to 4 AAs. Although their distributions vary from one organ to another, they are markedly expressed by the epithelia of intestine and kidney

mediating critical physiologic functions – important for the cellular homeostasis of peptides and AAs. PepT1 seems to be a key transporter in the intestine for overall AAs/peptide absorption only after high dietary protein intake.[156] Importantly, in the renal proximal tubule, there exist active transport mechanism(s) for small peptides, which are responsible for conserving the peptide-bound amino nitrogen otherwise it can be excreted through the urine. Further, the activity of these transporters seems to be orchestrated with the functionalities of the low-affinity peptide transporters in the lysosomes. This may indicate that the digestion and absorption of the dietary proteins and also peptidomimetic drugs may not be complete by intralysosomal digestion of proteins within intestine. Thus, the cellular digestion of peptides continue to generate free AAs and small peptides by the lysosomes, which display some other transporters such as AA transport systems in the lysosomal membrane. Similarly, some evidences have been reported upon the selective transport of di- and tri-peptides across the lysosomal membrane.[157,158] It should be also highlighted that the endoplasmic reticulum (ER) carries a transport mechanism for peptides in antigen-presenting cells. This is done by the functional expression of transporter associated with antigen presentation (TAP), where TAP1 and TAP2 build a functional heterodimer that mediates the translocation of the peptides across the ER.[159,160]

PepT1 can be used for peptidomimetic oral drug delivery through intestine.[161] Accordingly, the bioavailability of prodrugs specific to PepT1 was shown to be higher than those of the corresponding parent compounds, in large part because of the improved transportation. Such phenomenon have emerged a new field of peptide transporter mediated targeted therapy, so-called PepT1-targeted prodrugs.[162] Valganciclovir, a valyl ester of ganciclovir, was clinically shown to possess markedly higher bioavailability than ganciclovir when taken orally by patients with cytomegalovirus infection, in large part for being substrate of both PepT1 and PepT2.[163] A number of drugs seem to be substrate to these transporters, including amoxicillin, benazepril, captopril, cefixime, cephalexin, enalapril, levodopa, oxacillin, Ramipril, tolbutamide and valaciclovir. They are relatively stereoselective, while showing greater affinity to the peptides formed from L isomer amino acids than those possess D isomer amino acids. Kinetically, depending upon the substrate, PepT1 is considered as a low-affinity and high-capacity system which yields apparent affinity constants, K$_m$, ranging from 0.2-10 mM. However, PepT2 is also considered as a high-affinity and low-capacity transport system showing K_m values of 5–500 μM.

Endocytosis, transcytosis and RMTs

Cellular trafficking of macromolecular biomolecules is mediated through membranous vesicular machineries that are comprised of numerous components including lipid rafts, membranous caveolae and CCPs/clathrin-coated vesicles (CCVs). Various cell micro-/nano-

machineries are involved in vesicular trafficking, including Rab GTPases. Of various Rab family of proteins as a member of the Ras superfamily of monomeric G proteins, different Rab GTPases are responsible for regulation of different processes of the membrane trafficking such as the formation and travelling of vesicles within the cytoskeleton's actin and tubulin networks and even invagination and fusion processes. The newly biosynthesized protein in ER is trafficked across the Golgi (cis, medial and trans sections) to be matured and reach to other cell compartments or outwardly to the ECF through exocytosis, during which Rab GTPases play key roles. Fig. 8 shows schematic representation for the vesicular trafficking.

The cellular machineries involved in transportation of macromolecular biomolecules vary depending on the nature of the cargos and their interaction with plasma membrane. Endocytic internalization follows either nonspecific routes or specific mechanisms. Of the nonspecific endocytosis, the fluid-phase endocytosis (the so-called pinocytosis) is responsible for internalization of solutes that are present within the ECF bathing the plasma membrane, during which the plasma membrane encircle the surrounding solutes in the ECF and internalize them through the lumen of the budding vesicles. Classic fluid-phase markers include horseradish peroxidase (HRP) and dextrans.[164] In the case of nonspecific interaction of solutes through non-specific mechanisms such as electrostatic interactions, they are internalized by adsorptive endocytosis. Positively charged substances (e.g., cationic lipids, polymers, dendrimers, nanoparticles, and albumin) are able to interact with the cell surfaces that are negatively charged and internalize through the adsorptive endocytic path.[166-171]

The specific endocytic path is entirely dependent upon the receptor–ligand pairing process, in which solute molecules bind to its cognate receptor on the cell membrane. Once paired with the canonical receptor, solute are internalized into the cytoplasm through either a constitutive (class I) or ligand-stimulated (class II) internalization processes.[164] In the constitutive endocytosis, the continual plasma membrane turnover with the receptor internalized in an endocytic vesicle mediates the endocytosis process in the presence or even absence of the ligands such as Tf or LDL. In the ligand-stimulated endocytosis, the ligand-receptor pairing process is the main driving force for the initiation of the internalization of the ligands such as insulin and epidermal growth factors (EGFs).

In the RMTs, several key receptors are specifically responsible for the transportation of various biomolecules including insulin, insulin-like growth factor, leptin Tf and LDL.

Endosomal trafficking

Vesicles, after reacting into the cytoplasm, must be sorted out and directed to their specific itinerary. As a result, in a short period of time (<10 min), the internalized vesicles fuse with the early endosomes that act as an initial sorting station and express molecular markers such as the early endosome antigen-1 (EEA-1). In the early endosomes, where the pH is acidic (<pH 6.0), the transported cargo molecules dissociate from their specific receptors. This process is essential step for transportation of ligands to lysosomes for their degradation. The acidic condition of lysosomes enhances the binding affinity of IgG molecules in attaching to the neonatal Fc receptors (FcRns) that are expressed by variety of cells in particular endothelial cells such as BCECs,[172] and rescue the IgG molecules from degradation.[164] In polarized cells such as BCECs, the endocytic activities occur both in luminal and abluminal sections with distinct specifications that is in favor of either blood-to-brain or brain-to-blood traverse of substances, depending on biological prerequisites and activities of the brain cells. Such process also favors the recycling process of the endocytosed receptors, which allow cells to constantly recycle the receptor and dispose them back in the original membrane, unless they possess necessary signals for degradation or transcytosis. For example, the epidermal growth factor and its cognate receptor (EGF-EGFR) complex is basically trafficked to the lysosomes to be destructed, while other ligand-receptor pairs may possess signals for transcytosis. Trafficking of albumin following binding to its cognate receptor gp60 results in transcytosis of albumin.[173,174] FcRn-IgG complex undergoes transcytosis at the BBB in both directions, i.e., apical-to-basal (A-to-B) and basal-to-apical (B-to-A).[175] The B-to-A transportation (the so-called reverse transcytosis) of IgG molecules seems to depict the efflux of therapeutic immunotherapies from CNS in rat brain,[176] resulting in failure of the treatment modalities in CNS diseases. It is now clear that FcRn play a critical role in controlling IgG pharmacokinetics as well as serum albumin trafficking,[177] by which the improved pharmacokinetics of albumin-conjugated therapeutics can be explicated. In a study, to investigate the FcRn impact of the efflux of Abs, Cooper et al. treated rats with two mAbs specific to FcRn with markedly different binding affinities (IgG1 N434A and H435A) by intra-nasal-to-CNS and intra-cranial administrations. Upon intra-nasal delivery, mAbs reached the low cerebral hemisphere within 20 min, where the Ab with higher affinity to FcRn (N434A) showed a faster clearance trend. Having bilateral injection into the rat cortex, decreased and increased levels of N434A was detected respectively in the cerebral hemispheres and in serum, indicating the profound role of FcRn in efflux of IgG from the rat brain.[176]

In tack of selective retrieval of the endocytosed substances, they undergo further vesicular trafficking towards the late endosomes that are multivesicular bodies enriched in lysobisphosphatidic acid and others in phosphatidylinositol 3-phosphate (pH, 5.5–6.0) and potential to have homotypic/heterotypic fusion with lysosomes.[178] This is a process by which the endocytosed cargo molecules undergo the proteolytic degradation by lysosomes (pH, 5.0) that is known by the expression of the lysosome associated membrane protein-1. Once in the
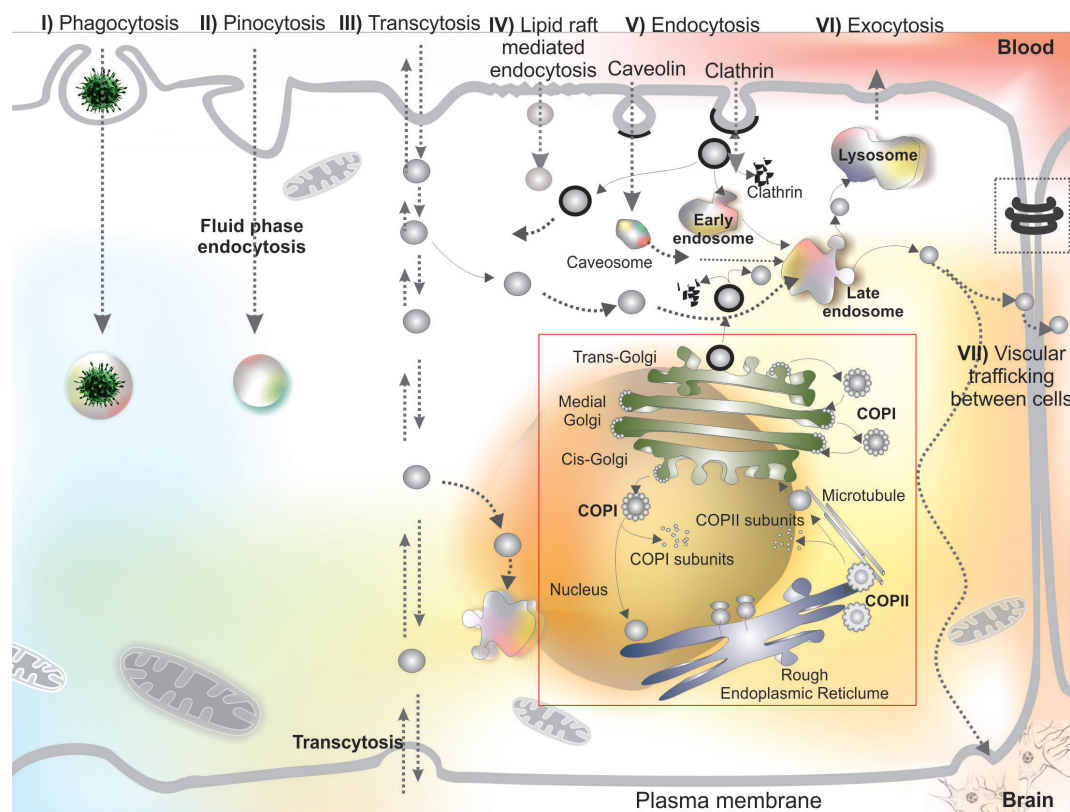
Fig. 8. Schematic representation for the diversity of vesicular internalization and trafficking. Macromolecular entities (e.g., bacteria, viruses) can be internalized through phagocytosis (I). Engulfed particles within the phagosomes are prone to fusion with lysosomes. Cells can internalize surrounding substances by a phenomenon called pinocytosis/fluid phase endocytosis (II). Transcytosis is the main route for crossing of macromolecules through cells (III). Lipid rafts play a key role in signal transduction and macromolecules (e.g., cholera toxin, glycophosphatidylinositol (GPI)-anchored proteins and SV40 viral particles) internalization (IV). Endocytosis can occur through membranous caveolae and clathrin coated pits (V). The clathrin mediated endocytosis can be categorized as coated pits endocytosis (transferrin (Tf), low-density lipoprotein (LDL), some toxins, free fatty acids and viruses) and cholesterol-dependent clathrin-mediated endocytosis (e.g., toxins such as tetanus and anthrax toxins, PRPC, IL-2 receptor and APP). Once internalized, depending on the type vesicle, it can interact with other cellular compartments or vesicles such as endosomes and lysosomes and exocytose (VI) or even interact with the neighboring cells (VII). In the case of newly synthesized proteins, the secretory proteins such as glycoproteins are basically traversed across the membranes of endoplasmic reticulum (ER) and cis-face of Golgi apparatus through coat proteins (COP) I/II. The matured peptides/proteins traverse through the cisternae and transporter out of the cells through vesicular trafficking from trans-face of Golgi apparatus (red box). During such biosynthesis, maturation and post translational modification processes in the ER and Golgi apparatus, the sorting machineries get involved by recruiting a number of adaptor, retrieval and coat proteins such as and soluble N-ethylmaleimide sensitive attachment protein receptor (SNARE). These bio-elements are implemented for the fusion of vesicles (see Fig. 9) and various Rab GTPases. For detailed information on vesicular trafficking readers are referred to the following citation.[134,164,165]

lysosome, the endocytosed molecules are exposed to array of hydrolytic enzymes such as proteases, lipases nucleases, glycosidases and phospholipases.[179-181]

For the endosomal trafficking, RMT follows different routes such as membranous caveolae, CCPs and lipid rafts that will be further discussed.

Caveolae-mediated endocytosis

Membranous caveolae are flask-shaped invaginations of the plasma membrane is described as "smooth" vesicles with sizes ranging from 50 to 100 nm and coated by a 22 kDa structural protein caveolin, whose N and C termini remain cytoplasmic.[182] Initially detected in endothelial cells, caveolae tend to mediate the selective uptake of various small molecules and macromolecules such as albumin and lipoproteins. Functional expression of

caveolin is linked with the maintenance of health and progression of various diseases such as cancers, hence it is considered as a new molecular marker for the targeted therapy of related diseases.[183,184] Caveolae has initially been detected in endothelial cells and is highly enriched in biomolecules such as glycosphingolipids, cholesterol, sphingomyelin, and lipid-anchored membrane proteins. Of these biomolecules, cholesterol plays a crucial role of in the biogenesis of caveolae. It has been validated by treating cells with cholesterol binding agents (e.g., nystatin, filipin, or cyclodextrin). This results in obliteration of caveolae structures. Further, the subcellular transition of the main protein of caveolae vesicles, caveolin, is intricately linked to the transportation of cholesterol. Given the fact that cholesterol is predominantly transported to the plasma membrane through a Golgi-independent vesicular
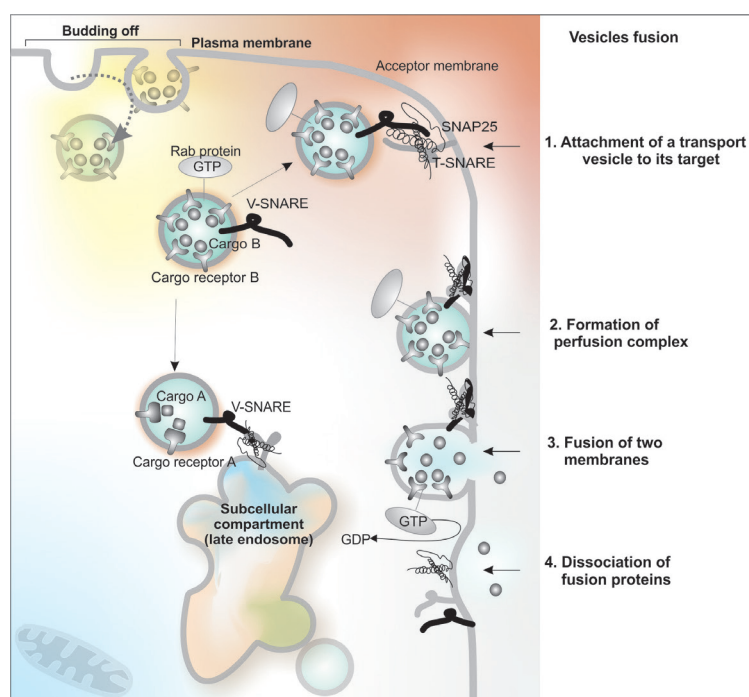
Fig. 9. Vesicular fusion through the SNARE complex and GTP hydrolysis.

path,[185,186] and the high binding capacity of cholesterol to caveolin-1, caveolae vesicles appear to be the main mediator of the cholesterol transportation.

It seems to elicit similar effect(s) on the biogenesis formation of the clathrin-coated endocytic vesicles and synaptic vesicles. It should be noted that the caveolin-1 is capable of forming extremely robust detergent-resistant homo-oligomeric complexes (comprised of ~14 to 16 monomers) that can further create higher oligomer-oligomer complexes through interactions with the respective C-terminal domains – a process in favor of bending of the plasma membrane similar to that of the triskelion in the CCPs.[182] Depending on the cell/tissue, the caveolae show different morphology such as grape-like clusters (abundant in developing skeletal muscle cells), rosettes (adipocytes), and detached vesicles/tubules in endothelial cells, as well as fused form as elongated tubules or transcellular channels.[182] Caveolae-mediated vesicles are involved in endocytosis, transcytosis and potocytosis. While for the endocytosis, caveolae bud off from the plasma membrane and fuse with various intracellular compartments such as caveosomes interaction with ER, during the transcytosis, caveolae vesicles deliver proteins from one side to the other side of endothelial cells. As for the potocytosis (cellular drinking), caveolae vesicles are involved in the uptake of small solutes (<1 kDa) such as folate, in which the vesicles are pinched off and remained associated with the cell membrane, then undergo the caveolar fission/fusion through the SNARE complex and GTP hydrolysis (Figs. 8 and 9).

While some recently conducted investigations implicated that caveolae and caveolin-mediated vesicles plays a check-and-balance role in the cerebral ischemia pathophysiology through incorporation with cholesterol molecules,[187] it can be molecular target for treatment of these diseases. Further, the upregulation of caveolin in various malignancies make it an important cancer marker,[188] while the endocytic capacity of the caveolar vesicles make this vesicular transportation route as an attractive mechanism for the targeted therapy, nonetheless caveolin-1 has been reported to be downregulated in some tumors. This vesicular mechanism has been shown to be beneficial path for macromolecular trafficking as reported for the endocytosis of atorvastatin calcium loaded BSA (ATV-BSA) nanoparticles (NPs), polyethylenimine (PEI), polyamidoamine (PAMAM) polyplexes and silica-coated iron oxide NPs.[189-191]

Clathrin-mediated endocytosis

Clathrin-mediated endocytosis (CME) is considered as the main RMTs process. During this vesicular trafficking phenomenon, CCPs/CCVs containing cargo molecules bud off from the plasma membrane and enter into the cytoplasm. Fig. 10 shows schematic illustration and transmission electron microscopy micrograph of the CCV. CME, as the most widely investigated vesicular transportation system, is a key step not only for the uptakes of nutrients and substances, but also for the intracellular signaling and membranous recycling. It should be noted that the binding of the receptors to the ligand molecules and activation of the receptors demand binding of intracellular part of the receptors to the adaptor proteins (Fig. 10A).[192] Adaptor proteins play central roles in the assembly of the clathrin protein onto the plasma membrane.[192] Of these, AP2 (subunits, α, β2, σ2, and µ2,) is one of the well-studied adapter proteins, which has a

number of binding partners such as phosphatidylinositol 4,5-bisphosphate (PIP2), clathrin, different endocytic accessory proteins, and two signaling motifs present on some cargo receptors (Fig. 10). The G-protein-coupled receptors (GPCRs) interact with various ligands (e.g., ions, amines, peptides, proteins, lipids, nucleotides, etc.), at which the receptor is activated with certain conformational changes towards plausible conversion of the receptor to the protein kinase substrate. The latter process results in binding of β-arrestins to the receptor, which is a necessary step for the internalization of cargo molecules through GPCR-mediated AP-2 adaptor protein complex and assisted by CME.[192,193]

Structurally, as shown in Fig. 10 (panel B), the clathrin-mediated vesicles is characterized by three distinct layers as (i) the inner layer in which cargo molecules are interacted with their cognate receptor in association with adaptor protein, (ii) the internal layer consisting of lipid bilayer interacted with integrated proteins, and (iii) the outer layer containing triskelion assembled as the
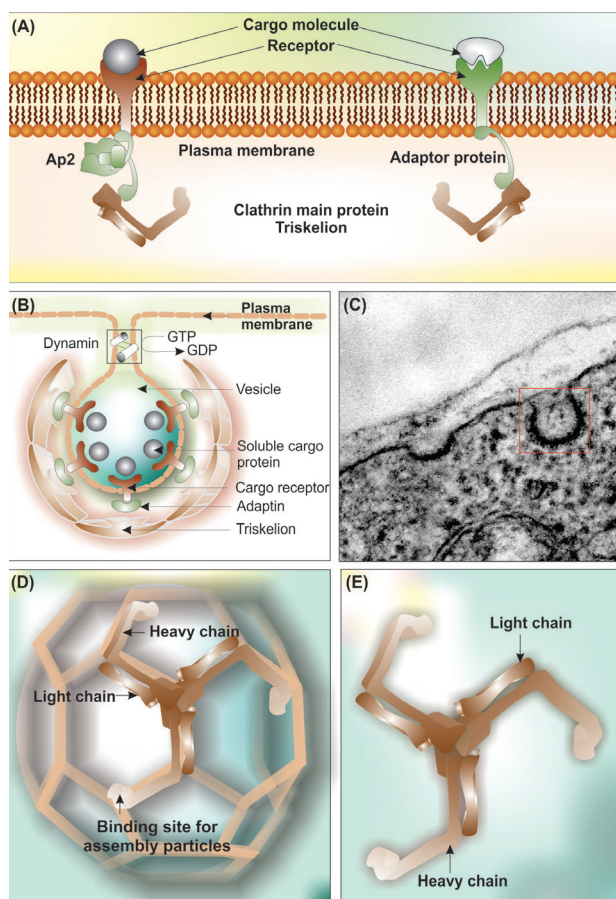

Fig. 10. Clathrin coated pits and clathrin-mediated endocytosis. (A) Schematic representation of plasma membrane interaction with cargo receptor, adaptor proteins and clathrin. (B) Clathrin forms a non-covalently bound triskelton structure composed of three heavy chains (192 kDa each) and three light chains. (C) The transmission electron microscopy micrograph of clathrin-coated pit in the brain capillary endothelial cell. (D) Schematic representation of assembled triskelion heavy and light chains forming pentagonic and hexagonic structure. (E) Triskelion heavy and light chains. Note: Not drown to scale.

pentagons and hexagons.

In general, the CME begins with (a) the interaction of cargo molecules with their cognate receptors, (b) the formation of vesicles on the inner surface of the cytoplasmic that possesses clathrin elements, (c) involvement of the AP2 adaptor protein complex and accessory proteins.

It should be highlighted that the subunits of AP2 adaptor complex initiate the formation of the clathrin matrix, in which some helper proteins (e.g., β-arrestins) are also involved. Once the α-subunit of AP2 attached to phosphatidylinositol-4,5-bisphosphate, it binds to the designated endocytic motifs through phosphorylation of μ2-subunit that results in binding of the μ2-subunit to the cargo protein undergoing endocytosis. All these events materialize the formation of CCVs, after which other accessory proteins such as clathrin assembly lymphoid myeloid leukemia (CALM) are literally involved to amalgamate the formation of clathrin lattice.[194] Concurrent with the emergence of clathrin matrix, various other proteins (dynamin and the actin cytoskeleton, rab5 guanine nucleotide exchange factor, Rme-6) are engaged to control the CCVs budding off, trafficking and recycling.[195,196]

CME participates in vesicular trafficking of blood-borne macromolecular substances into the brain by specialized functions at the BBB. Accordingly, the functional macromolecular trafficking through CCVs is important phenomenon not only for the maintenance of CNS homeostasis, but also for the targeted therapy of brain diseases such as brain tumors. For example, OX26 mAb has been recruited for crossing the BBB, which is a RME mechanism implementing TfR-mediated CCVs. Accordingly, various OX26 functionalized NPs have been developed including solid lipid NPs, PEGylated liposomes and polymers such as chitosan and poly(lactic-co-glycolic acid).[197-204]

Transcytosis

As a well-known transport process, transcytosis was first introduced in capillary permeability studies. In addition to the paracellular entry of the macromolecules through normal or anomalous opening of the biological barriers (Fig. 2), transcytosis is the main route for the entrance of macromolecules into the tissue/organ from the surrounding biological barrier. As shown in Figs. 2 and 8, transcytosis is an important process through which the cargo molecules move from one side of the cells to the other side bidirectionally (i.e., A-to-B and B-to-A).

In the brain microvasculature endothelia, at least three mechanisms are involved in entry of the particulate substances into the brain, including:

(i) caveolae, in which the pits (50–100 nm and flask shape) formed plasma membrane (Fig. 8) enriched with cholesterol and sphingolipids similar to that of the lipid raft components, then get coated with flexible caveolin-1/2 and bud off from the membrane by the aid of GTPase dynamin to carry cargo molecules (e.g., folate receptor, tetanus toxins and

alkaline phosphatase),

(ii) clathrin, in which spherical pits (60–200 nm) bud off from plasma (Fig. 10) by the aid of AP2 forming semi-rigid matrix that is cleaved by the GTPase dynamin to transport cargo molecules (e.g., LDL, LRP1, transferrin, insulin); and

(iii) micropinocytosis, in which irregular spherical/tubular vesicles (0.2-5 mM) perturb from the plasma membrane and are provoked by inflammation/lipopolysaccharide and shuffle various macromolecular entities.[27]

At the BBB, up to 20 receptors have been recognized with ability to initiate receptor-mediated transcytosis, including: TfR, heparin-binding EGF, scavenger receptors AI, BI, EGF receptor, tumor necrosis factor, insulin and insulin-like growth factor receptors, apolipoprotein E receptor 2, leptin receptor, melanotransferrin receptor, LDL receptors. Most of these receptors are associated with CCVs to initiate transcytosis.[27] Therefore, CCVs have been the main route for targeted delivery of nanoparticles and nanoconjugates into the brain.[197,201,205-209]

Multifunctional nanomedicines for brain targeting

While the currently used treatment modalities for brain diseases target the symptoms solely as noncurative agents, the nanoscaled DDSs show promising clinical outcomes by facilitating the drug delivery into the brain and improving the pharmacokinetics profile. However, these strategies need further investigations prior to their translation into clinical applications in human subjects. In fact, we need to improve our trifling understanding upon possible inadvertent neurotoxicity and detrimental effects of nanosized DDSs on neuronal cells. Targeting the cells within the central dogma brain is quit important for several formidable diseases such as neurodegenerative diseases and brain tumor gliomas. The latter disease has been classified into three major classes of astrocytomas, oligodendrogliomas and mixed gliomas (oligoastrocytomas), which cause a great deal of mortalities worldwide. Indeed, the targeted nanomedicines (e.g., polymeric/lipidic biodegradable NSs and inorganic nanoconjugates armed with targeting devices such as Tf or folic acid), with capability to cross BBB through active targeting mechanisms, can offer great benefits to patients with brain tumor.[210] In 2014, Su et al. capitalized on a dual-targeting DDS drug delivery system using bovine serum albumin nanoparticles (BSA-NPs) modified with both lactoferrin (Lf) and mPEG2000. The engineered NPs were loaded with DOX molecules and examined in both in vitro and in vivo models. While the in vivo pharmacokinetics analysis in healthy rats showed longer circulation in rat, the PEGylated Lf-armed DOX-loaded NPs induced profound toxicity in BCECs and C6 cells. As a result, the engineered NS was proposed as an effective targeting therapy against brain gliomas.[211] Given that Baicalin poses remarkable neuroprotective impacts on ischemic tissues in neurodegeneration, Liu et at. (2015) engineered PEGylated cationic solid lipid

NPs loaded with Baicalin and decorated with OX26 Ab (OX26-PEG-CSLN) with high encapsulation capacity (>80%) at a size range of about 30-50 nm.[198] Based on the pharmacokinetics studies, they showed significantly higher AUC value of OX26-PEG-CSLN (11.08-fold higher) than that of the Baicalin solution alone, concluding OX26-PEG-CSLN as a promising NS for targeting brain disease.[198]

In a study, brain-targeted peptide angiopep-2 (ANG) was used for surface decoration of poly(lactic-co-glycolic acid) (PLGA) NPs encapsulating DOX and anti-EGFR siRNA. This multimodal NS was found to efficiently deliver DOX and siRNA into U87MG cells, causing marked cytotoxicity and apoptosis and suppressing EGFR. Once used in the animal, the analysis of brain orthotopic U87MG glioma xenograft mice revealed that the ANG-armed PLGA NS was able to prolong the life span of the glioma-bearing mice.[205] Clark and Davis decorated Gold NPs (AuNPs) with Tf through an acid-cleavable linkage or anti-TfR Ab. They showed high affinity and avidity of Tf-armed AuNPs to TfRs on the blood side of the BBB. Once internalized, the multidentate Tf-TfR interactions was easily dissociated in large part because of acidification during the vesicular trafficking. Such mechanism was shown to profoundly enhance the entrance of NPs into the brain parenchyma of mice. While these researchers confirmed the trafficking of high-affinity anti-TfR Abs through lysosomal compartments of BCECs, they showed that the high-avidity Tf-armed AuNPs with the acid-cleavable linkage was able to avoid the major endothelium retention in large part through shedding surface Tf during their transcytosis.[212]

Recently, an "autocatalytic" approach was projected to enhance the transportation of NPs into the brain based upon entrance of a small number of NPs and liberation of specific modulators with capability to mediate the entry of more NPs into the brain.[213] The autocatalytic brain tumor-targeting poly(amine-co-ester) terpolymer NPs was used with results showing their ability to preferentially highly accumulate in brain tumors though through a passive targeting mechanism.

In 2014, Zong et al. developed a dual-targeting liposomal NS loaded with DOX and conjugated with cell-penetrating peptide (TAT) and Tf. Having examined in both in vitro and in vivo models, these researchers showed significantly high penetration of the dual-targeting NS across BCECs and marked accumulation in tumor cells. Based upon their findings by in vivo imaging, the engineered NS was able to reach the core of the tumor spheroids, resulting in marked increase in survival time of tumor-bearing mice after treatment in comparison with free drug molecules.[214] Another study was later on conducted to investigate the effectiveness of similar NS in targeted delivery of anti-EGFR siRNA to tumor bearing mice.[215] And, substantially long survival period and great downregulation in the expression of EGFR was reported in mice treated with such NS. As a result, it seems that the Tf-TAT conjugated NPs may be considered as a promising NS for shuttling drugs/

genes into the brain. In fact, various biological capacities of BBB such as CMTs and RMTs can be exploited for the specific targeting of brain diseases using different strategies.[216]

Concluding remakes

The BBB selectively/specifically controls/regulates the traverse of blood-circulating substances into the brain and vice versa, by which the CNS homeostasis is conservatively maintained. Such biological barrier also selectively controls the entrance of medicines necessary for diagnosis and/or therapy of the CNS diseases. The CMT and RMT machineries of the BBB can clinically be of great importance not only in terms of pathobiology of brain capillary endothelial cells and surrounding parenchymal cells, but also for drug discovery, delivery and targeting. In fact, our current challenge in CNS dreg delivery dilemma is how to exploit the naturally occurring vesicular trafficking systems and the receptor-mediated transfer machinery in safely crossing the BBB. We may also consider focusing our endeavors on exploiting multifunctional nanoscaled drug delivery systems (DDSs) to efficiently cross the restricted barrier and safely carry out the designated diagnostic or deliver therapeutic medicines to the target site(s) within the brain without imposing undesired impacts.[217] There have been a large number of investigations on developing smart multimodal brain drug delivery and targeting systems. While discussion on this subject was beyond the objective of this review, to the best of our knowledge, such DDSs are yet to be developed. Although multifunctional nanomedicines are supposed to be able to enter the brain exploiting the endocytic machineries mainly through functionalized ligands, their safety and nonspecific impacts on the brain parenchymal entities yet to be fully evaluated.

Ethical approval
There is none to be disclosed.

Competing interests
The authors like to declare that JB, MR and YO are the current editors of the journal. The publication process (blind peer-review) of the current article has been performed in accordance with the COPE guidelines.

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Review Highlights

What is current knowledge?
√ Brain capillary endothelial cells (BCECs) form blood-brain barrier (BBB).
√ BBB selectively/specifically controls the inward and/or outward traverse of nutrients/substances through carrier- and/or receptor-mediated transport machineries of BCECs.

What is new here?
√ Carrier- and/or receptor-mediated transport machineries of BCECs can be exploited for rationalized drug discovery and development.
√ For targeted therapy of brain diseases, the capacity of carrier- and/or receptor-mediated transport machineries of BCECs can be exploited.

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