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Structure of the Blood Brain Barrier and the Role of Transporters in the movement of substrates across the barriers

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Abstract

The blood brain barrier present in brain capillaries constitutes an essential barrier mechanism for normal functioning and development of the brain of structural integrity besides neuronal function. The structure and function of the BBB are summarised besides the physical barrier formed by the endothelial tight junctions, and the transport barrier resulting from membrane transporters and vesicular mechanisms. The presence of tight junctions between adjacent endothelial cells restricts the permeability and movement of molecules between extracellular fluid and plasma. It is divided into luminal and abluminal where each solute must cross both membranes. The roles of the neurovascular unit are outlined, especially the astrocyte endfeet, pericytes, and microglia. Five different systems of facilitative transport are found in the luminal membrane and are specific for a few substrates. Nonetheless, two major facilitative carriers (System L and y^+) are located in both membranes asymmetrically. In contrast, several Na^+ dependent transport systems transport amino acids against its concentration gradient present in the abluminal membrane, where the sodium pump Na^+/K^+-ATPase is highly expressed. The trojan horse mechanism is also favoured in drug delivery by employing molecular tools to bind the drug and its formulations. In the current work, we have revised the prevailing knowledge on the cellular structure of the BBB and the transport systems present exclusively for each substrate, and the need to find transporters with modifications that facilitate the transport of various drugs. Nevertheless, the blending of the classical pharmacology with nanotechnology needs to be focussed on promising results to rule out the BBB passage for the new generation of neuroactive drugs.

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Introduction

The central nervous system communicates both electrically and chemically being restricted to signal by neuronal and blood-tissue barriers. Blood tissue barriers are of several types including CSF blood barrier, blood cerebrospinal fluid barrier, blood neural tissue barriers (blood retinal and blood spinal cord barriers), and finally blood brain barrier. CSF blood barrier covers the ventricle surface completely in the middle part of the brain and is composed of avascular arachnoid epithelium located exactly below the dura surrounded by arteries and the circle of Willis. **Fig 1** Choroid plexus of epithelial cells comprises of blood cerebrospinal fluid barrier, whereas blood brain barrier consists of endothelial cells in the brain.[1][2] The blood brain barrier surface area comes in about 150 to 200 cm^2 /gram of brain tissue depending upon the anatomy region, accounting for 12 to 18 m^2 gross surface area for an adult human.[3]

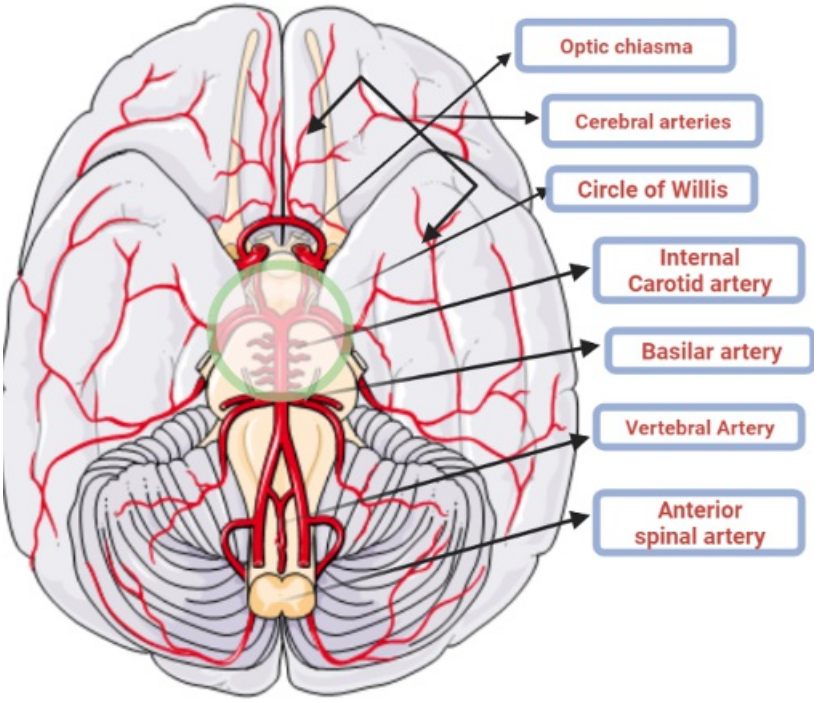

Fig. 1. *Cerebral arteries of the brain with internal structure of the brain showing the circle of Willis with other parts connecting the brain to the spinal cord.*

The term blood brain barrier was first introduced by Lewandowsky and co who injected the dyes (Trypan blue, Prussian blue) through intravenous route and observed that dye had little or no effect on the CNS but had stained other organs of the body indicating that BBB prevents substances from entering the brain.[4][5] The endothelial cells of the CNS along with pericytes and astrocytes establish the primary components of BBB. The property of the barrier is retained by dynamic and

continuous communication with the cellular elements of the neurovascular unit. Generally, BBB acts as a protective layer of the brain by shielding the direct exposure to blood borne pathogens and maintains the regulation of homeostasis in the brain microenvironment.[6][7] Additionally, it is also responsible for regulating ionic balance, nutrients, and macromolecules.[2] Although BBB acts as a protective barrier for the brain from several unwanted cells, it restricts therapeutic efficacy and leads to dreadful challenges in the development of new drugs for treating neurodegenerative diseases and brain cancer. The BBB acts as a diffusive barrier which is essential for the normal function of the central nervous system. The endothelial cells of BBB differ from endothelial cells in the rest of the body by the absence of fenestrations, sparse pinocytic vesicular transport, and more extensive tight junctions. The highly selective nature of BBB leads to exclusion in large molecules therapeutics and more than 98% of small molecule drugs happen to reach the brain safely[8]. Accordingly, it is to bring into the spotlight the development of a novel drug delivery approach, which delivers the therapeutics effectively to the brain without altering the normal structure and function of BBB. During the last decades, various strategies for delivering therapeutic substances to transport drug molecules beyond the blood brain barrier have been demonstrated.[9] Modulation of the tight junctions with several physical or chemical stimuli could potentially enhance the efficient process of drug transport but too much of this stimulus can affect the function of the brain adversely.[10] Drug molecules which are small i.e., below 500 Da can cross BBB easily by modifications of drugs through the lipidation process and limiting its wide usage and availability.[11] Nonetheless, the Trojan horse strategy in transporting drugs through BBB is defied because of its highly selective nature towards BBB.[12] Moreover, owing to the presence of multidrug resistance protein like P-glycoprotein in the luminal plasma membrane, the presence of an ATP-dependent efflux pump returns the drugs to the blood side besides successful penetration into the epithelium of the brain barrier.[13]

The immune privilege site like the brain with its barrier restricts the immune cell entry inside the brain.[14] The blood brain barrier with immune privileged function serves as microvasculature in the central nervous system, with its exclusive property involved in regulating the transport of iron, cells, and molecules between CNS and peripheral circulation. Dysfunction of BBB alters the homeostasis of cytokines, immune cells, and inflammasome in CNS ensuing in disorder of neurons and degeneration, which forms features in the pathology of Alzheimer’s disease (AD).[15][16] The interruption in the integrity of BBB is a hallmark of Alzheimer's disease in clinical research.[14][17] and entails that AD pathology is induced by leaky BBB. This integral loss of BBB leads to transport of inflammasome, cytokines, immune cells, and cytokines and disrupts the homeostasis further progressing to Aβ deposit, hyperphosphorylated tau, and expression of Apolipoprotein E.[18] Latest evidence suggests that BBB dysfunction is linked with an accretion of neurotoxic and vasculotoxic molecules resulting in a decline of cerebral blood flow and hypoxia.[19][20] Nonetheless, the argument also arises that leakage in BBB could be the effect of AD rather than the cause of AD.[21]

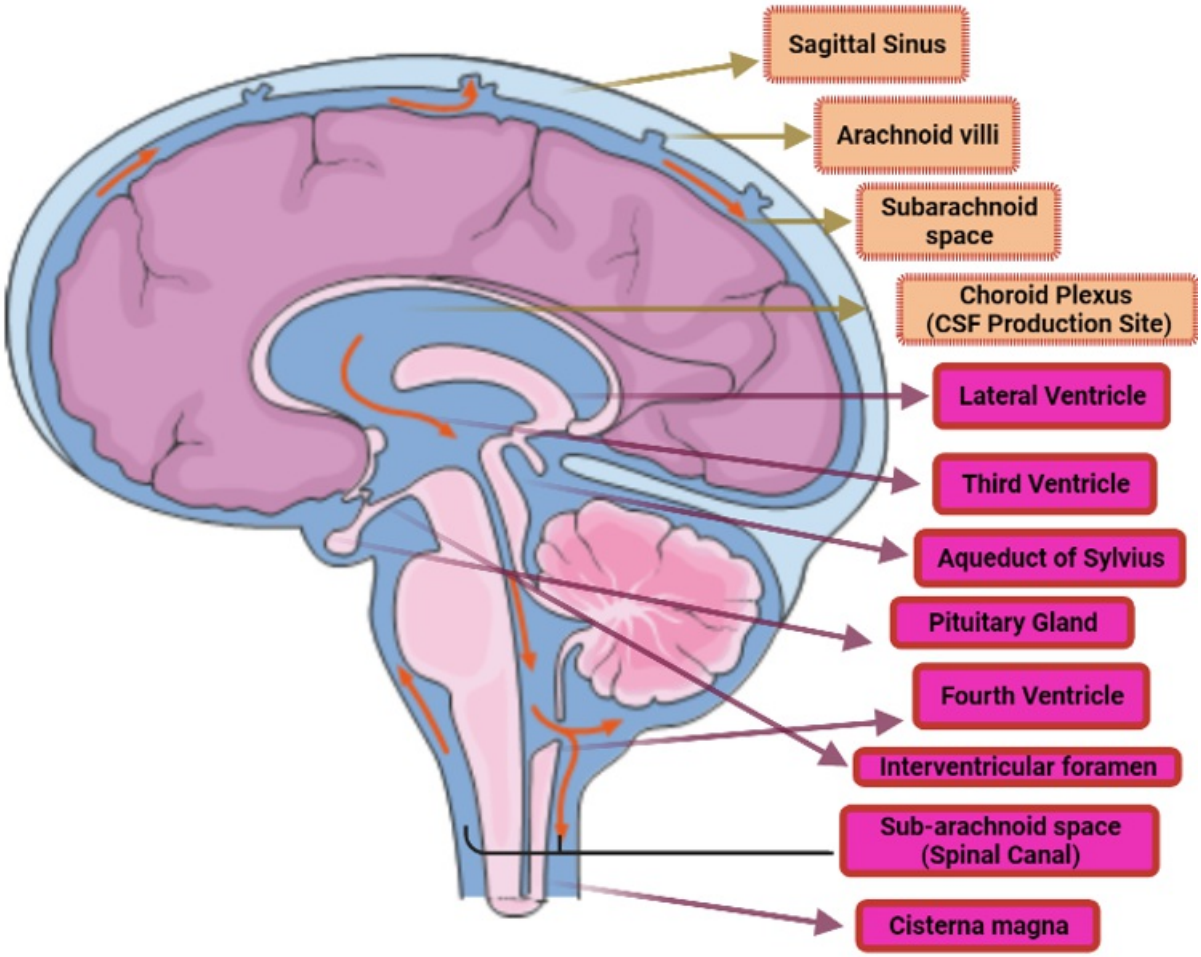

Fig. 2. Depiction of normal Cerebrospinal fluid flow in the brain across the ventricles.

Neurons communicate using a combination of signals both chemically and electrically with explicit management of the local ionic microenvironment around synapses and axons for reliable neural signalling[22] The First interface is formed by the combined surface area of these microvessels constituting the largest interface for blood brain exchange,[3] The second interface is constituted by epithelial cells of choroid plexus facing the cerebrospinal fluid forming the blood cerebrospinal fluid barrier,[23] and The third interface provides avascular arachnoid epithelium underlying the dura and completely enclosing the CNS and seals the extracellular fluids of the central nervous system with the rest of the body[24]. Despite the arachnoid membrane forming a barrier layer, it does not provide a surface area for exchange between CNS and blood owing to its avascular nature and relatively small surface area.[25] The barrier function at three interfaces is due to a combination of physical barrier, transport barrier, and metabolic barrier. The physical barrier has tight junctions between cells to reduce flux through intercellular cleft or paracellular pathways while the transport barrier shows the specific mechanism of transport mediating the flux of solutes, and the metabolic barrier will metabolize those molecules which are in transit with help of enzymes. The barrier function can be modulated and regulated physiologically and by pathology and is not fixed.[24]

Pacchioni granulation most found in lateral recesses of the superior sagittal sinus plays a significant role in the resorption

of cerebrospinal fluid and produces calvarial impressions between 13- and 15-mm lateral to midline in the region. This granulation can also protrude into the sinus lumen directly adjoining the venous entrance site and not to be mistaken for sinus thrombosis.[24] The capability of a compound to permeate BBB is estimated by measuring the blood-to-brain distribution, log BB, which is defined as the logarithm value of brain/plasma concentration ratios at equilibrium. Although Proteins have been proposed as potential therapeutic molecules to treat various disorders of the brain such as neuroinflammatory, neurovascular, and neurodegenerative diseases, they face many challenges which make them ineffective as a therapeutic agent, particularly when given through systemic route. Although systemic parenteral delivery is not the choice for achieving high protein concentration in CNS, it is most convenient clinically and used widely. The Hindrances in targeting the proteins in the brain include unfavourable pharmacokinetic properties of exogenous proteins like rapid serum clearance and/or degradation. According to Lipinski's Rule of 5, poor membrane absorption and permeation include charged molecules, hydrophilic, larger molecules, and hydrogen bond formation which suits for protein rendering it to prevent from conduction for passive diffusion through transcellular and paracellular pathways.[26][27] It is reported that molecules having hydrodynamic diameters larger than 11 Å and molecular weight of 500 Da are too large to pass through blood brain barrier.[28][29] Therefore, many developments are being made to achieve efficient drug delivery for larger molecules like proteins and effort in understanding the pathology leading to neurological disorders particularly those related to alteration in blood brain interface which provides a challenge in promoting sustained and remedial delivery.

The neurovascular unit comprises specialized microvascular endothelial cells with basal lamina covered with pericytes, astrocyte end feet, smooth muscular cells, neurons, and an extracellular matrix[30] The restrictive behaviour of the barrier comes from its complexity in structure by brain capillaries and endothelial cells express various active efflux transport proteins and tight junctions are the most restrictive in the body comprising three integral membranal proteins claudin, junction adhesion molecules, occludin, and accessory proteins like zona occludens ZO-1, ZO-2, ZO-3, cingulin, and so on.[31][32] The spatial arrangement/ positioning of these scaffold proteins affects the anchoring to the cytoskeleton as well as downstream signalling events regulating junctional dynamics. BBB has also additional enzymes which can degrade compounds and thus prevent their accumulation in the brain or enhance their elimination. Thus, various molecules which cross the cerebral endothelial cells are thus made in contact with ecto and endo enzymes present within endothelial cells containing a high density of metabolically active organelles like mitochondria. Additionally, endothelial cells of the brain show low pinocytotic activity and are separated by non-neuronal cells on the brain side (Pericytes) placing additional restrictions on its permeability.[33] The permeability and its selectivity in molecules at the choroid plexus membrane are strongly associated with the (BCSFB) Blood cerebrospinal fluid barrier. **Fig 2** The choroid plexus is vascular in nature localized around cerebral ventricles. The functional unit of choroid plexus tissue at the blood side contains leaky fenestration and highly permeable capillaries bordered by a monolayer of epithelial cells linked by tight junctions and facing cerebrospinal fluid.[34] Thus, any molecule crossing/ passing the endothelium should pass by the epithelium to reach the cerebrospinal fluid. The choroid plexus is roughly one hundred-fold leakier than the BBB, based on its electrical resistance and on lanthanum ion transport experiments.[35] Thus, besides its active production and secretion of CSF, antioxidants, and metabolism of drugs, it also develops a substantial surface area for exchange to happen.

Contrary to most structures of the brain, capillaries present in circumventricular organs lack tight junctions and do not exert barrier properties and it is also observed that there is a lack of Glut1 at the capillary endothelium of circumventricular organs[36][37][38]. Blood vessels present in a certain area of the brain have fenestration which permits the diffusion of blood-borne molecules across the vessel wall. These unprotected areas regulate the autonomic nervous system and endocrine glands of the body. Markedly, epithelial cells of choroid plexus possess both Tight junction and Adherens junction. Claudin 1,2,11, occludin, and ZO-1 are present in the epithelial TJs of choroid plexus whereas Claudin 1,5,11, occludin, and ZO-1 are present in the epithelial TJs of BBB.[39] Hence, claudin 2 and 5 are the only difference in the molecular composition of the Tight junction between the choroid plexus (BCSF barrier) and the BBB.

Recently, Parallel Artificial Membrane Permeability Assay has emerged as a promising technology which applies artificial membranes, non-biological to focus on the prediction of transcellular, and passive absorption.[40] The most significant parameter is brain penetration which shows its significant role in chemical toxicology studies and in drug design. In order to achieve the therapeutic potential, Penetration of the BBB by drug molecule targeting a receptor in the brain is highly essential.[41] To contrary, drugs not targeting CNS, the permeability of BBB could result in undesirable side effects. During Recent times, neuro-glio-vascular unit (functional unit composed of cellular constituents) comprising of endothelial cells, astrocytes, microglia, pericytes, myocytes, and neurons besides extracellular matrix is been observed[42] All such elements contribute to making cerebral capillaries, a physical and metabolic barrier and regulating the exchange between the blood and the CNS to ensure the maintenance and homeostasis of the brain.

BBB structure and transport routes

Neurovascular unit

The barrier is well developed, comprising microvasculature, astrocytes, endothelial cells, pericytes, and other components like smooth muscle, microglia, basement membrane, and neurons which exerts its role in immune function. Both endothelial cells and associating cells together maintain an intact barrier to bestow proper functioning of the central nervous system also known as the neurovascular unit[43] **Fig. 3**

Endothelial cells

The basic building blocks of the blood brain barrier endothelium layer is Endothelial cells forming a thin layer by connecting them with tight junction enabling tight connection as 50–100 times tighter than endothelial cells at the peripheral micro-vessel wall.[44] As an effect, intercellular junctions between the endothelial cells of blood brain barrier have no fenestration even when treated with a vascular endothelial growth factor[45]. Furthermore, blood brain barrier has few pinocytic vessels unlike endothelial cells in the rest of the body due to which small molecules or ions like glucose or

iron can be transported across the barrier with the assistance of enzymes (active transport).[46] Such Enzyme assisted process (Active transport) of nutrients from blood to the brain requires greater energy potential than the diffusive transport occurring in the endothelium of other body parts. Another interesting fact is that endothelial cells of blood brain barrier have around 5 to 6 times more mitochondria per capillary section than that of skeletal muscle capillaries[47], and it provides excess energy for active transport across blood brain barrier. Besides endothelial cells of blood brain barrier, it also possesses an enzymatic barrier due to its proteolytic enzymes like alkaline phosphatase, c-glutamyl transpeptidase, and aromatic acid decarboxylase which exert the capacity to break down the neuroactive bloodborne drugs and solutes[45]

Contrary to peripheral endothelial cells, those present in CNS are more prompt in regulating ion transport, macromolecules, and the cells present in between blood and brain.[48] The two-fundamental division of transporters for permeability in barrier in endothelial cells include efflux transporters and the highly specific nutrient transporters, where the former is located on the luminal surface for transporting diversified lipophilic molecules into the blood and the latter transports specific nutrients across the brain barrier into the CNS and remove the toxic substances from CNS into the blood.[49] Endothelial cells require more energy to retain selective permeability of the brain barrier and their active transport function which supports the fact that dysfunction of BBB will increase with age. The layers of the endothelial cells determine the permeability of molecules. Distinct layers of endothelial cells are present in large arteries and veins, whereas a single layer of endothelial cells facing the lumen of vessel is present in the smallest capillaries of the CNS.[50] Whilst, the single layer and enormous surface area of endothelial cells in CNS contribute to the performance of the brain barrier, and also make it vulnerable. Once endothelial cells get damaged or dysfunctional, the risk for disorder in the brain enhances dramatically due to loss in blood brain barrier partially. Various studies were assessed in a mouse model of Alzheimer's disease and found that microcirculation dysfunction is correlated with impairment of endothelial cells before Amyloid β accumulation.[51] Moreover, *in vitro* studies have exhibited that a higher concentration of Amyloid β promotes the degeneration of endothelial cells and limits the formation of new capillaries. This anomaly affirms results from in vivo studies that Amyloid β inhibits angiogenesis and suppresses the formation of blood vessels.[52]

Pericytes

The word peri means around and cyto means cell which establishes the peri-endothelial location at the basal side of the microvessels. It exists between the endothelial cells and astrocyte endfeet. The contractile mural cells (Pericytes) moderately cover the endothelial cells of blood brain barrier and form two basal laminas -BL1 and BL2 along with the smooth muscle.[53] Among the basal laminas formed, BL1 is the definite extracellular space between endothelial cells and pericytes, while BL2 is the extracellular space between pericytes and the glial endfeet bounding the brain parenchyma[54] Furthermore, the permeability and function of blood brain barrier are determined by the covered pericytes around endothelium as permeability increases with insufficiency if pericyte coverage[55] Additionally, pericytes also have other functional aspects like BBB-specific gene expression, strengthening of tight junctions, the polarization of astrocytes end-feet, and vesicle trafficking.[56] Henceforth, the interaction between endothelial cells and pericytes is crucial for

regulating blood brain barrier whose failure leads to dysfunction of the barrier and neuroinflammation.

The presence of RAGE, LDLR, LRP1, and CD36 Aβ receptors in the layer of pericytes advocates that pericytes are involved in the development of Alzheimer's disease.[57] Deficiencies in the pericytes begin accelerating the development of Alzheimer's by inducing deposits of amyloid plaques.[58] The pericytes are also contained by APOE4 through the CypA-NFκB-MMP9 pathway and damage the barrier of the brain by curbing the production of tight junctions.[58] The pericytes also activate the innate immune system by regulating LFA-1, ICAM-1, and Mac-1 based on the ability to recruit immune cells. It is also regulated by pro-inflammasomes such as CXCL1,8, CCL2, and IL-6 in AD development but its role is still unclear.[59]

Astrocytes endfeet

Astrocytes are star shaped glial cells in the CNS which forms complex network through their end-feet surrounding the endothelial cells and basal lamina, which links up the endothelial cells with microglia and neurons which is fundamental for the proper BBB properties and functions.[60] The astrocyte endfeet surround the blood vessels in the brain and influence the integrity of BBB by illustrating the significance of astrocytic endfeet transporters. The morphology is complex due to its contact with a huge number of dendrites and several synapses. Astrocytic endfeet preferably regulate the diffusion of molecules in the extracellular space 43 One of the crucial functions is the water transport between the CNS and the peripheral circulation and regulating Ca^{2+} level.[61] Data suggests that endothelial cells of the brain cultured with astrocytes are less vulnerable under different pathologic conditions and promote anti-oxidative activity of the BBB, which is critical to protect the BBB against oxidative stress[62]. Furthermore, astrocytes also enhance the level of tight junction proteins by expressing pentraxin 3 and inhibits the pericyte differentiation by binding with integrin A2 receptor through the brain deriving specific basement membrane protein (Laminin)[63] Additionally, astrocytes have scaffolding property, homeostasis, and injury protection, and clearing of synapses which is considered as the primary workhorse of the CNS[64] Astrocyte endfeet enriched channel protein, AQP4 limits the rate of water transport owing to high composition of lipids in BCSFB and BBB.[65] Nonetheless, when astrocytes retract their endfeet from the cerebral vessels, the permeability of blood brain barrier is enhanced.[66] Astrocytes are well studied for immunological function and ability to perform autophagy in cognitive degenerative diseases such as AD. Moreover, astrocyte endfeet are impaired by hyperphosphorylated tau protein and oligomer Aβ.

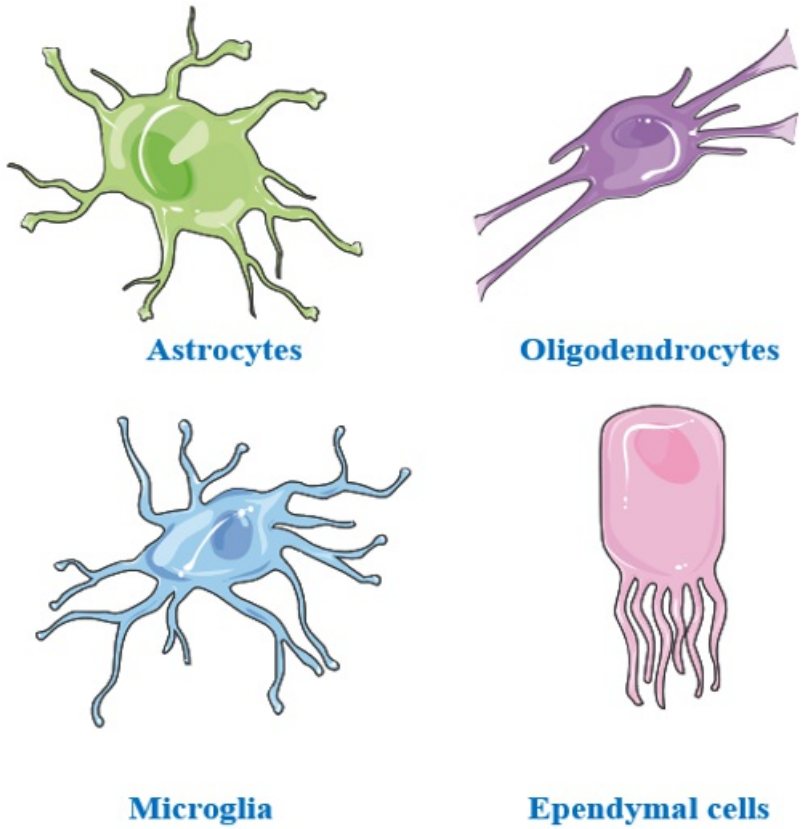

Fig 3: Neuroglia comprising of Astrocytes (Regulates transmission of synapse and protect neurons from toxic compounds also provides an anti-oxidant activity by releasing precursor of Glutathione to neurons and controls neuroinflammation), Oligodendrocytes (Provide insulation to the axons of nerve fibres by myelinating similar to the function performed by Schwann cells in the peripheral nervous system for conduction of signals), Microglia(Regulates Homeostasis of CNS by removing unwanted synapse and neurons, mediates immune response and perform phagocytosis by acting as macrophages), and Ependymal Cells(Involved in regulation and production of CSF which is circulated around the CNS by the layer of cilia present on the apical surfaces and lines the CSF filled ventricles).

Claudin

Claudin is a 22 kDa phosphoprotein with four transmembrane domains which is one of the major components of tight junctions and is present exclusively at the strands of tight junctions as conceded by immune replica electron microscopy. Claudins from one endothelial cell bind to the claudin of another endothelial cell homotypically to form primary seal of tight junction.[67] The carboxy terminal of claudins binds to cytoplasmic proteins including ZO-1, ZO-2, and ZO-3![68] In the brain, claudins-1 and 5 along with ccluding are said to be present in endothelial tight junctions forming the BBB.[69] Claudin 11 (oligodendrocyte protein) is a major component of CNS myelin. Loss of claudin 1, but not claudin 5 from cerebral vessels was observed under pathologic conditions like tumor, stroke, and inflammation.[70]

Occludin

Occludin is a 65-kDa phosphoprotein larger than claudin and shows no amino acid sequence similar to claudins. It has four transmembrane domains, a long COOH terminal cytoplasmic domain, and a short NH_2 terminal cytoplasmic domain. The two extracellular loops of ccluding and claudin originating from neighbouring cells form the paracellular barrier of Tight junctions. The cytoplasmic domain is directly associated with ZO proteins.[71] Occludin expression is much higher in endothelial cells of the brain compared to non-neural tissues. Occludin appears to be a regulatory protein that can alter paracellular permeability.[72] Claudins and occludins interact and form heteropolymers to form intramembranous strands which are visualized in freeze fracture replicas. These strands contain certain fluctuating channels which allow selective diffusion of ions and hydrophilic molecules.[73] The concomitant loss of a 55-kDa ccluding expression results in the breakdown of BBB in the tissue surrounding the brain tumor.[74]

Junctional adhesion molecules

Junctional adhesion molecules (approximately 40 kDa) belonging to the immunoglobulin superfamily[75] have a single transmembrane domain and its extracellular portion has two immune globulins like loops formed by disulfide bonds. Three JAM-related proteins, JAM-1,2,3 have been investigated in the brain section of rodents. It was observed that JAM-1 and 3 are expressed more in blood vessels of the brain but not JAM 2.[76] The expression of JAM in BBB of humans is yet to be explored and is involved in cell-to-cell adhesion and transmigration of monocyte through BBB.[77] Nevertheless, our knowledge of the function of JAM is incomplete, and a more detailed study is required to unfold its function in the BBB.

Cytoplasmic accessory proteins

Cytoplasmic proteins involved in the formation of tight junctions include zonula occludens proteins -ZO-1,2,3, cingulin, 7H6 etc. ZO-1,2, and 3 have a molecular weight of 220 kDa, 160 kDa, and 130 kDa with sequence similarity among each other and belong to membrane-associated guanylate kinase-like protein (MAGUKs). It contains three PDZ domains - PDZ1,2,3, one SH_3 domain, and one guanyl kinase-like domain. These domains act as protein binding molecules and play a key role in organizing proteins at the plasma membrane. The PDZ1 domain of ZO-1,2,3 has been reported to bind directly to the COOH terminal of claudins.[78] Occludin interacts with the GUK domain on ZO-1![79] JAM was also shown to bind directly to ZO-1 and other PDZ-containing proteins recently.[80] Significantly, the primary cytoskeleton protein (actin) binds to the COOH-terminal of ZO-1 and 2, and this complex cross-link transmembrane elements and provides structural support to the endothelial cells.[81]

Other components of BBB

Among the components of BBB other than astrocytes, and pericytes include microglia, and basement membranes. The

vascular basement membrane in CNS wraps the smooth muscle and pericytes and separates the endothelial cells from neurons and glial cells contributing to vessel formation and guaranteeing the integrity of BBB[82] The monocyte lineage cells like microglia are located around the brain and spinal cord and comprise 5–20% of the total glial cell population in the brain parenchyma[83] Being the resident macrophage cells, two major functions- immune defense and CNS maintenance are performed[84] Moreover, increasing evidence indicates that tight junctions of blood brain barrier can be modulated by those activated microglial cells which enhance the integrity and function of BBB.[85] Hence, the barrier of BBB is regulated and maintained by crossed communication between the cellular elements of the neurovascular unit. The tight connection between neighbouring endothelial cells is expedited by three distinct types of inter-endothelial cell junctions- Adherens junction, newly identified gap junction, and tight junction.

Tight junction

The tight junctions are formed by certain cytoplasmic proteins and transmembrane proteins where the former includes zonula occludins (ZO-1,2), cingulin, monoclonal antibody 7H6, calcium/calmodulin-dependent serine protein kinase (CASK), Afadin (AF-6), and latter includes claudins, junction adhesion molecules (JAMs), and occludins. The transmembrane protein like JAMs highly localized on the tight junctions of BBB is a member of the immunoglobin subfamily and expressed by platelets, leukocytes as well as endothelial cells and regulates polarity of the cell, migration of leukocytes, and permeability of the endothelium.[76] JAMs have two domains: extracellular domain and cytoplasmic domain in which the extracellular part coordinates the communication between endothelial cells and leukocytes by combining synergies of b1 and b2 integrins while the cytoplasmic part communicates with several tight junction-associated proteins viz., ZO-1 and AF-6[86] Furthermore, JAMs located on the endothelial cell surface can contribute to adhesive interactions with circulating platelets[87] Till date, four distinct types of JAMs are identified in the BBB as JAM-A, B, C, D which is primarily involved in homophilic interactions. For instance, JAM-A will interact with another JAM-A on other endothelial cell surface. Even though JAMs have their own advantage in creating tight junctions, occludins and claudins are also one of the most critical transmembrane proteins for tight junctions.[88] Occludins are type II transmembrane proteins with similar functions to claudins that are expressed in brain microvascular endothelial cells and exclusively localized at tight junctions.[89] Claudins are transmembrane proteins with four distinct types i.e., Claudin- 1, 3, 5, and 12 identified at BBB. The extracellular domain of claudins seals the paracellular cleft forming a tight junction between two neighbouring endothelial cells and the intracellular domain connects the filaments of actin through cytoplasmic scaffolding proteins. Cytoplasmic proteins which contribute to making an intact tight junction structure include monoclonal antibody 7H6 (which creates a link between scaffolding proteins) and the actin cytoskeleton. zonula occludens-1,2,3 are the intracellular scaffolding proteins connecting occludins and claudins to the actin filaments.[90] Zona occludens distribute its C-terminal end over the plasma membrane surface and other actin-rich structures to connect tight junction proteins with the actin filament whereas the N-terminal end links with the tight junctions' proteins, such as claudins and occludins[91] Besides Tight junctions, cadherins (adherence junctions) forms a junctional complex between the endothelial cells of brain 28 Studies has reported that Aβ1-42 is toxic to tight junctions and adherence junctions which suppresses the expression of claudin, occludin, cadherins, and JAMs which explains how dysfunctional barrier of the

brain contributes to the risk of Alzheimer's disease.[92]

Adherens junction

Adherens junction is composed of cadherin – catenin complex and its associated proteins. Cytoplasmic proteins link membranous proteins to actin (primary cytoskeleton protein) to maintain the structural and functional integrity of the endothelium. The BBB is present in all brain regions, except for the circumventricular organs including area postrema, pineal gland, neurohypophysis, median eminence, lamina aterminalis, and subfornical organ.[93] Additionally, BBB comprises capillary basement membrane, astrocyte end-feet ensheathing the vessels, and pericytes embedded within the Basal membrane. Pericytes (a cellular component of the BBB) though less extensively studied, has a key role in the structural integrity and differentiation of the vessel, angiogenesis, and formation of endothelial Tight junctions.[54] The most significant part of the blood brain barrier is the structural integrity and assembly of tight junction proteins which is performed by the Adherens junction. It is formed by cadherin, a transmembrane glycoprotein present at the basal side of cell-cell junctions in endothelial cells of BBB. Vascular endothelial cadherin (VE-cadherin/cadherin-5) is a homo-dimeric transmembrane protein that spans the paracellular cleft, where the extracellular domain of VE-cadherin of one endothelial cell forms a homo dimer by connecting to VE-cadherin molecules of neighbouring endothelial cells.[94] Paracellular cleft holds the cell altogether providing support to tissue. The cytoplasmic domain of VE-cadherin communicates with the actin filament through scaffolding proteins (p120, A, B, C-catenin.[95] Furthermore, A- catenin can mediate the interaction of B-Catenin with the actin cytoskeleton and C-catenin (plakoglobin) which in turn binds to the cytoplasmic domain of cadherin-5 to link the cadherin complex to the cytoskeleton[96] Other transmembrane proteins linked with the adherens junction are nectin, PECAM-1, and CD99. The specific roles of these associated proteins are still unclear. On the whole, the adherens junction is indispensable for the structural integrity of inter-endothelial cell connections and any such alterations on the junction lead to the disruption of blood brain barrier.[97] Adherin junctions perform homophilic interactions between the extracellular domains of calcium-dependent cadherin on the surface of adjacent cells and assemble. The cytoplasmic domain of cadherin binds to the sub-membranal plaque proteins (h- or g-catenin) which is linked to the actin cytoskeleton through A catenin. Various components including cadherin, vinculin (A-catenin analog), and alpha-actinin have been demonstrated in intact microvessels of the BBB in the rat. TJ and AJ components are known to interact, particularly ZO-1 and catenins, and influence TJ assembly.[98]

Gap junction

A gap junction is a space located in between the tight junction and the adherens junction. In chordates, it is an intercellular channel formed by the hexamers of medium-sized families of integral proteins- connexins, innexins, and pannexins[99] Three connexins have been identified in BBB as Cx37,40,43 among which Cx43 is the most ubiquitously expressed connexins in endothelial cells of the brain.[100] Each connexin can form a gap junction after oligomerization in the endoplasmic reticulum and homo/ hetero hexamerization at the plasma membrane. Connexins typically have four

transmembrane spanning domains with unstructured C, and N-terminal cytoplasmic tails, while the C terminal tail regulates gap junctions and the N terminal regulates oligomerization in the endoplasmic reticulum.[101] In blood brain barrier, gap junctions permit ionic exchange and small molecules throughout between adjacent endothelial cells. Additionally, gap junctions of blood brain barrier are responsible for the transduction of metabolic signals and also regulate the permeability of blood brain barrier by interaction with scaffolding proteins (ZO-1) through the linkage of afadin-6 protein.[102]

Basement membranes

Two basement membranes exist in CNS microvessels viz., endothelial basement membrane below the endothelial cells containing type IV collagen, fibronectin, and laminins 4,5, and astroglial basement membrane underlying the astrocytic endfeet comprising fibronectin, agrin, and laminins.[103] The basement membranes consist of an extracellular matrix; structural proteins construct the physical scaffolding for cells and aid in the synthesis of Tight junctions.[104] Similar to other structures in the brain barrier, the basement membranes are affected by the toxic form of Aβ. Collagen IV, perlecan, and fibronectin are decreased in patients with Alzheimer's disease. Laminins recruits circulating leukocytes into the brain during inflammation. Due to the devoid of transport proteins inside the basement membranes/ Tight junctions, they provide a passive defence mechanism and are impaired in the late AD rather than the initial period in AD or other CNS disorders.

Transport pathways across the blood brain barrier

Depending on the various physiochemical properties, the penetration of molecules into the brain may occur by the following routes (a) paracellular transport (small hydrophilic molecules) (b) transcellular passive diffusion (lipophilic and nonpolar solutes such as carbon dioxide and oxygen) (c) carrier-mediated transport by the diverse members of the solute carrier (SLC) family of transporters (d) adsorptive-mediated transcytosis (internalization of molecules following a nonspecific interaction) (e) receptor-mediated transcytosis (Using the receptors present on the cellular surface), and (f) cell-mediated transcytosis (deliver drugs by relying on immune cells). **Table 1** Polar solutes and macromolecules cannot diffuse across BBB and require other transport mechanisms. Even though BBB works as a barrier of molecular transport between circulating blood and the brain parenchyma, certain transport routes exist for transporting proteins and peptides to maintain the homeostasis of the brain.[105] The transport routes include diffusional transport in the form of transporter proteins mediated transcytosis, paracellular and transcellular transcytosis, adsorptive mediated transcytosis, receptor-mediated transcytosis, and cell-mediated transcytosis.[106] The driving force for Paracellular diffusion (non-specific transport mechanism) is the transport of solute molecules through a space between two neighbouring endothelial cells by the negative concentration gradient from blood to the brain. p-glycoprotein, a Multidrug-resistant protein, and breast cancer resistance proteins altogether limit the accumulation of various hydrophobic molecules and potentially toxic substances in the brain.[107] P-glycoprotein, an ATP-dependent efflux pump contributes to the efflux of undesirable substances such as amyloid-β proteins from the brain into the blood as well as anti-cancer drugs. The inhibition of P-gp

has both favourable and unfavourable effects. P-gp deficiency at the BBB induces the increase of A β deposition in the brain of an Alzheimer's disease mouse model.[108] It is also known that the A β deposition is inversely correlated with P-gp expression in the brain. However, the transient inhibition of P-gp by antidepressants enables medicines such as anti-cancer drugs to enter the brain.

These proteins also forbid the therapeutics accretion in the brain through two phases where the first phase collectively prevents the uptake of drug molecules by endothelial cells and the second phase actively expels out the anticancer therapeutics, such as vinblastine, daunorubicin, and doxorubicin from the brain. **Fig 4** It is alleged that ATP provides sufficient energy for the transportation of drugs against a negative concentration gradient[107] The efflux pumps present in BBB have both a positive and a negative impact as they are responsible for decreasing neurotoxic harmful effects of drugs and also restrict the therapeutic effect in CNS which is beneficial in treating neurodegenerative diseases. Thus, alteration of efflux pumps at the blood brain barrier might be a prospective approach to boosting the access of therapeutics into the brain and offers novel therapeutic options in various neurodegenerative diseases.[109] Another significant mechanism in the transportation of drugs across BBB is by utilizing the receptors on the surface of the cell a.k.a receptor-mediated transcytosis (RMT) and is used nowadays for transporting Nanoparticle-based drug delivery as it takes advantage of receptors expressed over the apical surface of the endothelial cells of the brain by endocytosis.[106] In this process of endocytosis, the ligand binds with the receptor specifically and they form an intracellular vesicle through membrane invagination. The most targeted receptors for RMT are lactoferrin receptors, transferrin receptors, diphtheria toxin receptors, insulin receptors, and low-density lipoprotein receptors. In receptor-mediated transcytosis, membranal invagination occurs either through clathrin or caveolae-mediated mechanism, where clathrin-mediated RMT forms a basket-like convex structure and caveolin-mediated invagination by forming endocytic vesicles.[110]

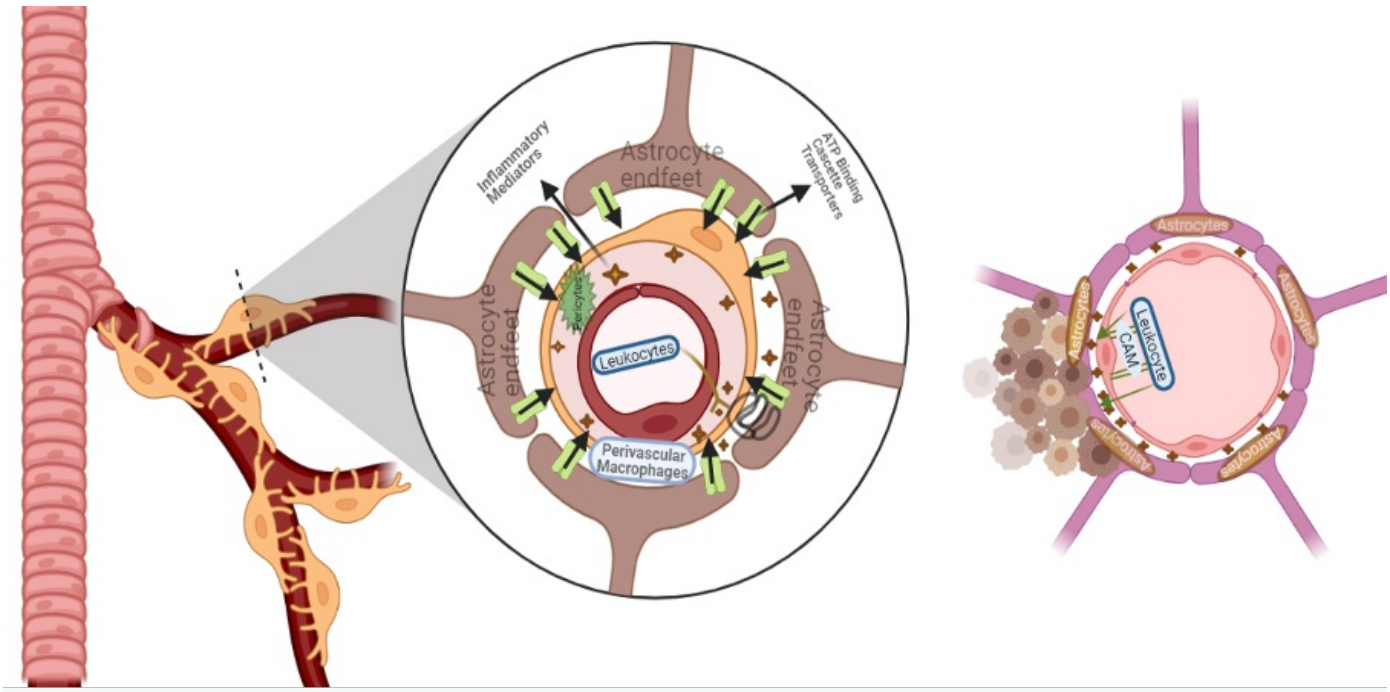

Fig 4. *The Figure indicating the Brain vascular system of BBB, formed by brain endothelial cells, surrounded by astrocyte endfeet, pericytes, and*

perivascular macrophages. Under normal situations, astrocytes express ABC transporters marginally whereas, in a diseased state, leukocytes adhere to brain Endothelial cells through cell adhesion molecules (CAM), which leads to migration of the cells into CNS causing damage to tissue. It is also instigated that reactive astrocytes highly increase the expression of ABC transporter and its function, which leads to enhanced efflux of inflammatory mediators (Star shaped). These mediators in turn may (1) increase endothelial CAM expression and subsequent leukocyte adhesion and (2) stimulate leukocyte attraction into the CNS via chemokine secretion.

The novel transporting mechanism like adsorptive mediated transcytosis (AMT) is utilized for transporting charged nanoparticles or macromolecules across BBB. It utilizes the advantage of induced electrostatic interactions between positively charged drug carriers and negatively charged microdomains on the cytoplasm membrane[111] As this AMT-mediated mechanism does not require a receptor surface, a larger number of particles can bind to a cellular surface with low binding affinity and allow therapeutic delivery in concentrated form but owing to its non-specific process it causes the accumulation of drugs in other organs. Additionally, another transport mechanism involved is by cell-mediated transcytosis (CMT-Trojan horse strategy) to deliver the drug across BBB and it relies on immune cells (Monocytes, Neutrophils, and Macrophages) which have the ability to cross BBB in both healthy and disease outcomes.[112] In this CMT technique, drugs are encapsulated in a liposome to absorb quickly by the immune cells of the circulating blood, which later cross the BBB and migrate towards inflammatory sites in the brain by using their unique properties called diapedesis and chemotaxis.

Table 1. Various Transporters and their substances transported

Transporters	Substances Transported	Function	Mechanism	Reference
Energy Transport system				
Sodium Independent Glucose transporter1, 3,4 (GLUT1,3,4) a.k.a SLC2A1-solute carrier family 2, facilitated glucose transporter 1	Glucose	This uniport protein Facilitates the diffusion of glucose across a membrane, and intracellular glucose metabolism is initiated by the glucose-phosphorylating enzyme (hexokinase IV or glucokinase)	Transcellular	113
Monocarboxylate transporter 1,2,4 (MCT1,2,4)	lactate, pyruvate, and ketone bodies	facilitate the transport of lactate and other endogenous monocarboxylates and therefore play an important role in cellular metabolism	Transcriptional as well as post-Transcriptional	114
peptides transport system				
PTS 1	Enkephalins/Tyr-MIF-1	leucine regulates the transport rate of peptide transport system-1 from brain-to-blood peptide transport in the abluminal membrane	The phosphoryl group on PEP is eventually transferred to the imported sugar via several proteins. The phosphoryl group is transferred to the Enzyme E I (EI), Histidine Protein (HPr, Heat-stable Protein), and Enzyme E II (EII) to a conserved histidine residue, whereas in the Enzyme E II B (EIIB) the phosphoryl group is usually transferred to a cysteine residue and rarely to a histidine.	115
PTS 2	AVP, Enkephalins	facilitates bidirectional transport of LHRH		116

PTS 3	Peptide T	The reverse process of transport on the luminal membrane transports Peptide T into the brain.		117
PTS 4	AVP, LHRH	PTS 2,4,6 is bidirectional transport of LHRH		118
PTS 5	NA	brain-to-blood peptide transport		NA
PTS 6	PACAP27	Retards the accumulation from the blood by the brain of the 27 amino acid form of pituitary adenylate cyclase-activating polypeptide (PACAP27)		119
PTS 7	NA	The reverse process of transport		NA
ATP binding cassette Receptors				
ABCB1/MDR1/P-gps	morphine, digoxin, verapamil, dexamethasone, saquinavir, nelfinavir, saquinavir, paclitaxel, loperamide, actinomycin D, Leukotriene C4, D4, E4,	ABCB1-type P-gp actively transports substrates against a concentration gradient.	ABC transporters must pump transport substrates against a chemical gradient, a process that requires ATP hydrolysis as a driving force. ABC transporters operate in a single direction (either import or export), although the drug efflux pump LmrA has been shown to be reversible under certain conditions	120,121
ABCC1/MRP1	sulfate conjugates, LTC 4, vincristine, daunorubicin, doxorubicin, etoposide, MTX, GSH, GSH conjugates, Glucuronide conjugates	expressed in non-P-gp expressing MDR cell lines and involved in the extrusion of conjugated xenobiotics that may be harmful to the brain	At the cellular level, in contrast to the apical membrane location of other ABC transporters, MRP1/ABCC1 is predominantly located in the basolateral membrane of polarized cells. Thus, MRP1/ABCC1 likely pumps its substrate into the interstitial space of the body, rather than excreting them into bile, urine, or gut.	122,123
ABCC2/MRP2	bilirubin, cisplatin, prastatin, sulforhodamine 101 acid chloride (Texas Red), GSH, GSH conjugates, glucuronide conjugates	Eliminates conjugates of various toxins and carcinogens with GSH, glucuronate, or sulfate from hepatocytes into bile, from kidney proximal tubules into the urine, or from intestinal epithelial cells into the intestinal lumen.	The molecular mechanisms by which ABCC2 is targeted to the apical membrane are incompletely understood.	124
ABCC3/MRP3	monoanionic and conjugated bile acids, MTX, etoposide, teniposide, and Organic anion transporter	localizes to the basolateral portion of the hepatocyte plasma membrane, where it transports glucuronide conjugates and bile salts from the cell and is inducible upon bile duct ligation. However, another transporter, the organic solute, and steroid transporter (OST) complex, has been recently found to be the major mediator of this process	Baasolaterally located MRP3 may allow efflux of organic anions from the liver into the blood when the biliary secretion is blocked. The mechanism is unclear now, but one possibility is genetic polymorphisms affecting MRP3 expression	125,126
ABCC4/MRP4	cyclic nucleotides (cAMP, cGMP), nucleotide analogs (PMEA, PMEG), purine analogs, prostaglandins, MTX, unconjugated bile acids, sulfate conjugates, GSH, glucuronide conjugates	active efflux transporter of anti-influenza virus drug oseltamivir. *ABCC4* expression levels decrease with the differentiation toward mature leukocytes which could promote cAMP accumulation and hence the strength of signalling down differentiation pathways.	MRP4 mediates the transport of various endogenous bioactive substances. Notably, several signalling molecules include cyclic nucleotides, prostaglandins, and leukotrienes. As mediators of the cyclooxygenase pathway, prostaglandins support an inflammatory microenvironment and can promote cell proliferation and survival in tumor cells, including neuroblastoma	127-129
ABCC5/MRP5	cyclic nucleotides (cAMP, cGMP), nucleotide analogues (PMEA), stavudine monophosphate, GSH	MRP5 is of particular interest for signal transduction. MRP5 has been shown to mediate the cellular efflux of 3′,5′-cyclic nucleotides, cAMP, and cGMP	It acts as an Alternative mechanism in the control of intracellular cyclic nucleotide levels, in addition to the well-established metabolic degradation by phosphodiesterases.	130
[illegible]	small peptides, etoposide, cisplatin,	*In vitro* studies supports that it functions as an organic anion efflux pump transporting	[illegible]	[illegible]

ABCC6/MRP6	daunorubicin, doxorubicin, GSH conjugates	(an) unidentified substrate(s) from the liver toward the circulation.	The mechanism is not clearly understood.	131
ABCG2/BCRP	doxorubicin, topotecan, methotrexate, imatinib, pitavastatin, cerivastatin, zidovudine, mitoxanthrone	*In vitro* studies have demonstrated that these TKIs (Tyrosine kinase inhibitors) are substrates/inhibitors of the efflux transporters P-gp and BCRP as well as the uptake transporter OCT1	The role of BCRP in drug resistance in cancers has not been well established. There are currently no clinical studies aimed at overcoming cancer drug resistance by inhibiting BCRP.	132
Organic anion/cation transport system				
Choline transporter-like protein 1 (CTL1)	Choline	The purpose of this transport is to provide choline for the synthesis of the neurotransmitter acetylcholine. Choline can also be transported by the members OCT1 and OCT2 of the organic cation transporter family with a low affinity.	Choline uptake studies have established CTL1/SLC44A1 as an Na^+-independent choline transporter at the plasma membrane of various cells and tissues. CTL1/SLC44A1-mediated choline transport appears to be linked to both phospholipid and betaine synthesis but might also mediate choline transport for non-neuronal acetylcholine synthesis	133
Organic anion transporter- 1,3 (OAT1/3)	Xenobiotics, PAH, Cyclic nucleotides, PGE2, Mercurials.	capable of the bidirectional movement of substrates, most of the Oats are generally viewed as facilitating the movement of organic anions into the epithelial cells (influx transporters).	Oat-mediated influx involves the exchange, or counter transport, with another solute (which for the prototypical Oats is believed to be α-ketoglutarate), and these transporters are thought to be part of a so-called “tertiary” transport system involving the organic anion transporter, the Na^+-K^+-ATPase, and the sodium-dicarboxylate cotransporter	134
Organic cation transporters 1-3 (OCT1/2/3)	Oxycodone, Prazosin, Amantadine, N-methyl nicotinamide, corticosterone, L-carnitine, pentamidine	Regulation of neurotransmitters in neurons rather than at the BBB. It has a role in direct CNS distribution from the nasal cavity to the brain	pentamidine is a substrate for OCT1 transporter at the BBB and is then effluxed by ATP-dependent mechanisms, most likely P-gp	135
Organic anion transporting polypeptide 2B1, 1A4 (OATP2B1, OATP1A4)-Bidirectional transporter	Dehydroepiandrosterone sulfate (DHEAS), a neurosteroid	play a major role in CSF detoxification by limiting the distribution of organic anions to the brain and spinal cord. It is localized both on the luminal and the abluminal membranes of brain capillary endothelial cells, thus has low permeability to BBB	Oatp1a4 is selectively localized on the basolateral membrane of rat choroid plexus epithelial cells, which form the blood-cerebrospinal fluid barrier, suggesting a role of Oatp1a4 in blood-to-CSF transport.	136
Organic cation/carnitine transporter 2 (OCTN2)	Carnitine	sodium ion-dependent transporters for the metabolism of fatty acids	OCTN2 behaves as an exchange transporter in Na+ -dependent carnitine reabsorption in the kidney and mediates Na+ -independent secretion of organic cations	137
Scavenger receptors				
SR-A	Glycated type IV collagen, proteoglycans biglycan, and decorin	Expressed at the ECs of cerebral capillaries. Multifunctional, multiligand pattern recognition receptor with roles in innate immunity, apoptotic cell clearance, and age-related degenerative pathologies such as atherosclerosis and Alzheimer’s disease.	SR-A was found to recognize the exchangeable apolipoproteins A-I and E in both lipid-free and lipid-associated forms, suggesting the shared α- helix as a potential recognition motif.	138
SR-B(I)	Cholesteryl ester	Receptors are less suitable for targeting drugs in the brain. play a role in the transport of cholesteryl esters at the BBB. Malfunction of this receptor can thus also result in atherosclerotic events leading to neurodegenerative processes in the brain.	In the systemic circulation, the binding of HDL to scavenger receptor BI (SR-BI) culminates in phosphorylation of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production that is dependent on the adaptor protein PDZK1	139,140
CD-36 (Fatty acid Translocase)	Fatty acids	Regulating normal physiological and pathological functions. CD36 pathways are activated by several distinct ligands. Convergence of these pathways results in inflammatory responses and endothelial dysfunction, which may be an underlying	CD36 recognizes membranes of cells undergoing apoptosis. Although CD36 responses occur in a ligand-specific manner, the pathways triggered by different ligands often converge, generating free radicals and inflammatory mediators within the cell, both inside and outside of the vasculature. The resultant inflammatory	141

		cause of cardio- and cerebrovascular diseases. It is also involved in lipid metabolism.	responses compromise endothelial function and the integrity of the blood brain barrier (BBB)	
Amino acid transporters				
Large endothelial amino acid transporter 1 and 2	Large neutral amino acids in sodium independent pathway, L-dopa, Gabapentin, baclofen, L-leucine, L-phenylalanine, and L-tryptophan	To maintain metabolic function and protein synthesis is mediated by system L (facilitative transporter) present on both sides of the brain i.e., luminal and abluminal due to sodium independent pathway	The carrier appears to operate principally via a sodium-independent, substrate-coupled antiport, although it can mediate net influx.	142,143
cationic amino acid transporters 1 and 3	lysine, arginine, and ornithine	It is mediated by System Y^+, a Voltage-dependent system where the Na^+-dependent transport system is present on the abluminal membranes.	CAT-1 is differentially expressed in tissues, including the brain, but does not appear to be enriched at the BBB. CAT-3 is highly expressed in the brain and readily discriminates between L-lysine and L-arginine based on affinity (a difference of approximately twofold).	143,144
Excitatory amino acid transporters 1,2,3	Glutamine	Luminal carriers of amino acids have no dependence on Na^+ gradients and are therefore energy-independent. Because the substrate was presented to the capillary lumen, it was deduced that these transporters are present at least in the luminal membrane. glutamate does not enter the brain in material quantities, except in the circumventricular organs	The Na^+-dependent transporters work at the limit of their ability to maintain the glutamate gradient between the brain cells and the ECF and, of course, the steep Na^+ gradient as well (extracellular >> intracellular) that is maintained by Na^+/K^+-ATPase. If the oxygen supply is insufficient to maintain ATP concentrations, membrane Na^+/K^+-ATPase ceases to function. Under these circumstances, the Na^+ gradient is dissipated and glutamate is released from both astrocytes and neurons by reversal of the EAAT family of transporters. If ECF glutamate rises, nerve cells may be damaged.	145,146
Neutral amino acid transporters 1,2 (ASCT1/2)	Alanine, Serine, Cystine, Glycine.	ASCT1 (SLC1A4) was observed in brain Endothelial cells during embryonic and neonatal development, but not in adult brains. ASCT2 (SLC1A5) is expressed at higher levels than ASCT1 on the abluminal side.	Na^+-dependent system located exclusively on the luminal membranes of the BBB	147-149
glycine transporter (GLYT1)	Glycine	Neurotransmitter clearance from excitatory synaptic clefts mostly relies on the glycine transporter GlyT1 located in neighbouring astrocytes, although lower levels of the transporter are present in glutamatergic terminals and in the postsynaptic membranes of glutamatergic synapses	GlyT1 belongs to the SLC6 transporter family that includes Na^+ and Cl^-dependent co-transporters for neurotransmitters, osmolytes, and amino acids.	150
Membrane Transport protein				
Major facilitator superfamily domain-containing protein 2 (MFSD2a)	lysophosphatidylcholine (LPC)-binded fatty acids	Mfsd2a regulates nutrient supply from the blood into the brain while mediating BBB integrity. Mfsd2a is selectively expressed in the blood vessel endothelium and may suppress vesicular transcytosis in endothelial cells in the central nervous system.	Mfsd2a regulated the BBB function via regulating vesicular transcytosis across the cerebral endothelium.	151,152
Na+ myoinositol transporter	Myo-inositol	SMIT1 might also be helpful against Alzheimer's. *SLC5A3*, encoding SMIT1, is in fact expressed in target regions for medication within the brain	The mechanism for the impact of altered SMIT1 expression is still under discussion. *Myo-inositol* depletion might lead to altered levels of phosphoinositides, which causes impaired vesicle trafficking and disturbed phosphoinositide-dependent signalling	153
H+ myoinositol symporter	Myo-inositol	HMIT expression appears highest in the hippocampus and cerebral cortex, areas which are implicated in mood disorders	HMIT is a symporter of myo-inositol and protons, since inositol uptake was only evident under acidic extracellular conditions and was associated with an inward electrical current and decreased intracellular pH	154
		SPNT (sodium-dependent purine		

Concentrative nucleoside transporter 2 (CNT2), SLC28A1.	purine nucleosides, uridine, adenosine.	nucleoside transporter). CNT2 is capable of transporting nucleoside-based anticancer and antiviral drugs. CNT2 is widespread in the brain, being most abundant in the amygdala, hippocampus, cerebellum, and certain neocortical regions.	the mechanism of the induction has not yet been clarified	155,156
Ion Transport System				
Sodium-dependent multivitamin transporter (SMVT)	Biotin, pantothenic acid, lipoic acid	SMVT has been recently gaining importance due to its low affinity and high capacity along with wide substrate specificity.	SLC5A6 is a Na^+-dependent multivitamin transporter (SMVT) that mediates cellular uptake of biotin, pantothenic acid, and lipoic acid	157
Na^+ Ca^{2+} exchanger	Sodium, Calcium	Regulation of intracellular Ca2+ concentration via the forward mode (Ca2+ extrusion) or the reverse mode (Ca2+ influx)	There are three isoforms of NCX in the brain, and they show developmental changes: the main isoform of immature rat brain is NCX1, whereas that of adult rat brain is NCX2	158
Voltage-gated K^+ channel (KV1)	The selective flow of potassium ions.	Kv1 channel plays an important role in cell proliferation and apoptosis.	In some disorders, such as in brain tumors (astrocytoma, oligodendroglioma, and glioblastoma) a clear correlation between the channel expression and the stage of the disease is still not established	159
Na^+K^+ATPase pump	sodium, potassium	Maintenance of brain water and electrolyte homeostasis.	Transport of Na+ and K+ across the blood-brain barrier is modulated by noradrenergic innervation from the locus ceruleus.	160
Na^+, K^+,2Cl^-co-transporter	sodium, potassium, chloride	mediates the coupled movement of Na+ and K+ with Cl^- across the plasma membrane of the cell. In the brain, NKCC1 is expressed in cortical and cerebellar neurons, glia, brain capillary endothelial cells, and epithelial cells of the choroid plexus. NKCC1 plays a critical role in regulating cell volume and ion homeostasis	The failure of the ATP Na+ /K+ pumps results in an increase in intracellular ionic content and an influx of water into the cells.	161
Transient receptor potential Channels/ Group A lipid gated Ion Channel (nonselective Ca^{2+}-permeable channels)				
Transient receptor potential channel TRPC-Canonical (Non-selective)	PIP2, DAG	TRPC3/6/7 group regulate diverse functions in neurons and glia	TRPC channels work as receptor-operated cation channels. opening of	162
Transient receptor potential channel TRPV-Vanilloid	THC, Capsaicin	In functioning as a thermosensor, the ion channels TRPV1 play a pivotal role in pain and energy homeostasis.	Activation of TRPV1-induced elevations in calcium concentration of the mitochondria, endoplasmic reticulum, and nucleus can cause damage to the cells, mediate cell death, and eliminate TRPV1-expressing cells	163
Transient receptor potential channel TRPP-Polycystin	AITC (Allylisothiocyanate)	physiological functions, ranging from pure sensory functions, such as pheromone signalling, taste transduction, nociception, and temperature sensation, over homeostatic functions, such as Ca^{2+} and Mg^{2+} reabsorption and osmoregulation, to many other motile functions, such as muscle contraction and vasomotor control.	TRPA1 channel undergoes a pore dilation coupled with an increased Ca^{2+} permeation and an increased fraction of Ca^{2+} contributing to the total current.	164
Transient receptor potential channel TRPM-	pregnenolone sulphate, ADPR, cADPR.	TRPM4 is sensitive to intracellular calcium ($[Ca^{2+}]_i$) levels and responsible for capillary fragmentation, resulting in secondary hemorrhage and a severe impact on neurological function. Besides, TRPM4 is highly susceptible to ATP depletion due to the persistent opening of	TRPM2 channel is induced by ROS, such as hydrogen peroxide (H_2O_2), nicotinamide adenine dinucleotide (NAD+), and related metabolic products including ADP-	165,162, 166

Melastatin		TRPM4 channels, which leads to oncotic swelling and oncotic death of endothelial cells through cellular depolarization and continuous influx of Na^+, resulting in capillary fragmentation	ribose (ADPR) and cyclic ADPR (cADPR)	
pericyte transporters				
Lipoprotein receptor1 (LRP1)	Hydrophobic/ Basic drugs	controls the endocytosis of a variety of ligands, influences signaling pathways by coupling with other cell surface receptors or proteins, and directly regulates gene expression through its intracellular domain.	LRP1 couples with the platelet-derived growth factor (PDGF) receptor and traffics into endosomes, where the phosphorylation of the PDGF receptor is induced in the presence of PDGF	167
Receptor for advanced glycation end products (RAGE)	Amphoterin, amyloid-β toxins	transports circulating amyloid-β toxins across the blood–brain barrier (BBB) into the brain. RAGE–amyloid-β toxin interaction at the BBB leads to oxidative stress, inflammatory responses, and reduced cerebral blood flow	Oligomer species appear more pathogenic. Aβ transport at the BBB, cerebral blood flow reduction, and neuroinflammation.	168
Clathrin-coated process				
transferrin receptor (TR)	Fe containing Tf (holo-Tf)	Fe containing Tf (holo-Tf) binds to the transferrin-receptor (Tf-R) and is transported into the ECs through the clathrin-mediated endocytic pathway.	The transport mechanism for all these targeting peptides and antibodies remains unknown	169
melanotransferrin (MTf), or the P97 protein, MF12, CD228	Iron	Transports iron independently from the Tf-R route. A role for MTf has been suggested in Alzheimer's disease because its expression has been shown in reactive microglia in amyloid plaques.	MTf is actively transported across the BBB, by receptor-mediated transcytosis at rates 10 to 15 times higher than those obtained with either serum transferrin (Tf) or lactoferrin (Lf)	170

Conclusion

The Blood brain barrier is the fundamental component of the central nervous system. Its functional and structural integrity is essential to maintain homeostasis of the microenvironment of the brain. Any disorder in the BBB function causes its secondary effects on cerebral blood flow, vascular tone, and influencing transport across BBB. Various proteins are involved in the maintenance of impermeability to systemic circulation. Despite numerous transporters available for transporting various substrates, each has its own limitations in developing an efficient target and drug delivery systems need to be addressed. The active transporters in the brain located at the abluminal membrane provide a mechanism to remove amino acids at higher concentrations which could be toxic. The Glutamine, being neurotoxic, is removed from the ECF of the brain through the most active transporters -EAAT present in astrocytes and glycine is removed by systems A and N. It helps in preventing the activation of receptors and alters the expression of efflux transporters/ disintegrity of tight junctions.

Declarations

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Conflicts of interest/Competing interests

The author declares no Conflict of interest

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