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Review Article

The blood-brain barrier: a focus on neurovascular unit components

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ABSTRACT

The blood–brain barrier (BBB) provides an optimum environment for neurons by ensuring the integrity and homeostasis of highly fragile brain cells under physiological conditions, protecting the brain from changes in the blood with both structural (tight junctions) and metabolic (enzymes) barriers, selective transport, and the metabolism and modification of substances in the blood and brain. The endothelial cells of the brain capillaries, located at the interfaces between the blood and the brain, are critical components that limit the permeability of the BBB. These cells have unique morphological, biochemical, and functional characteristics that distinguish them from those found in the peripheral vascular system. In addition to endothelial cells, astrocytic perivascular end-feet, pericytes, neurons, microglia, and smooth muscle cells also play significant roles in maintaining the homeostasis of the brain parenchyma. Thus, the BBB effectively prevents various molecules and therapeutic drugs from entering the brain parenchyma and reaching the target area at sufficiently high concentrations. The passage of a substance through the BBB and its entry into the brain depends on various factors, including the substance's lipophilicity, diffusion capability, molecular weight, electrical charge, blood concentration, and multiple primary and secondary factors. Drug delivery systems developed in recent years, through techniques and methods aimed at controlled and safe opening or bypassing of the BBB, are believed to provide significant benefits in the lesion area by allowing therapeutic substances to optimally enter the brain from the circulation. This article provides a review of the BBB and its components, highlighting their significance among the brain's different interfaces. It also discusses approaches for delivering therapeutic substances to the affected area under optimal conditions and concentrations in various brain pathologies.

Keywords: Astrocytic perivascular end-feet, blood-brain barrier, drug delivery, microglia, neurovascular unit

1. INTRODUCTION

In the central nervous system (CNS), neurons communicate through chemical and electrical signals. Therefore, for healthy neural signaling, precise regulation in the local ionic microenvironment of synapses and axons is essential [1]. During the regulation of molecular exchanges between blood, neural tissue, and the fluid-filled spaces within the brain, six interfaces play a role: the blood–brain barrier (BBB), the blood–cerebrospinal fluid (CSF) barrier, the meningeal barrier, the circumventricular organs, the adult brain ependyma, and the fetal neuroependyma [2]. In this review, various concepts related to the BBB, the prominent features and functions of the components constituting the BBB,

the transport systems involved in crossing the BBB, the limitation of drug passage into the brain due to the BBB's strong barrier properties in various brain diseases, and various invasive and noninvasive strategies developed to overcome this barrier and enhance therapeutic efficacy are examined.

2. COMPONENTS AND FEATURES OF THE BBB

The BBB is a dynamic interface that separates the brain interstitium from the luminal contents of the cerebral vascular system [3]. This interface maintains the integrity of highly fragile brain cells under physiological conditions, ensures brain homeostasis, and provides an optimal environment by protecting the CNS from various physiological and pathological changes. Instead of the commonly used term "Blood–Brain Barrier," some researchers in recent years have proposed the concept of "Blood–Brain Border" [4].

The neurovascular unit (NVU) is a complex functional unit that reflects the dynamic communication between the components of the BBB and neurons [5]. It is composed of microvascular endothelial cells surrounded by a basal lamina, astrocytic perivascular end-feet, pericytes, neurons, microglia, and smooth muscle cells [6,3]. Below is a brief overview of the components that make up the NVU and maintain the integrity of the BBB.

2.1. Endothelial cells

Microvascular endothelial cells are considered the anatomical foundation of the BBB due to their distinctive morphological, biochemical, and functional characteristics that set them apart from their counterparts in the peripheral vascular system [7,8]. These cells, which line the inner surface of brain capillaries in a single layer and are in direct contact with the blood, are characterized by being surrounded by the basal lamina and astrocytic perivascular end-feet, containing tight junctions (TJs) composed of transmembrane proteins such as claudin, occludin, and junction adhesion molecules, having very few pinocytic vesicles in their cytoplasm, and lacking fenestrations [9,10]. In addition, these cells have smooth, oval nuclei with irregularly distributed chromatin, caveolae, similar invaginations on the luminal side, and large quantities of mitochondria to enhance energy potential for enzyme and transport system activities. They are also equipped with specialized transport systems and receptors that facilitate the uptake of nutrients and hormones essential for brain function [8].

To eliminate gaps between endothelial cells and prevent the paracellular diffusion of substances from the blood into the brain parenchymal area, the endothelial cells of capillaries and postcapillary venules not only possess TJs, which provide primary isolation but also have adherens junctions. These junctions restrict the paracellular flow of hydrophilic molecules but do not limit the passage of small lipophilic molecules such as O_2 and CO_2. Thus, these molecules can diffuse freely across plasma membranes along concentration gradients. The diffusion of O_2 and CO_2 across the endothelium is emphasized as being essential for regulating brain metabolism and pH in NVU cells [11].

The BBB not only functions as a physical barrier but also acts as a metabolic (enzymatic) barrier due to the expression of numerous enzymes by endothelial cells that can modify a variety of molecules. These enzymes can either inactivate pharmacologically active drugs or activate inactive prodrugs [12]. These include L-amino acid decarboxylase, monoamine oxidase, glutamyl aminopeptidase, transaminases, and especially cytochrome p450 enzymes. It has been noted that some molecules with neuroactivity, such as neurotransmitters or drugs that could affect normal physiological brain functions are prevented from entering the brain by being enzymatically converted into inactive forms once they penetrate from the luminal surface of the capillary endothelium into the cytoplasm [13].

2.2. Basement membrane

The blood vessels of the CNS contain various basement membranes, including endothelial, astroglial, and meningeal. The astroglial and leptomeningeal basement membranes form the parenchymal basement membrane that defines the boundary of the brain parenchyma. In brain capillaries, the endothelial and parenchymal basement

membranes merge to form a single basement membrane [7]. The basement membrane, structured by brain microvascular endothelial cells, pericytes, and astrocytes, is made up of three adjacent layers composed of various extracellular matrix molecule classes, including collagen, elastin, fibronectin, laminin, and proteoglycans [14]. Thus, the brain endothelium is supported by the extracellular matrix and basement membranes, along with other cells of the NVU [15]. Although the basement membrane does not constitute an important barrier against the diffusion of small molecules, it has been emphasized that it plays key roles in anchoring cells and regulating the functions of endothelial cells through various signaling molecules [16]. The basement membrane can become thicker or thinner in response to stress stimuli and certain pathological conditions. The loss of the characteristic features of the basement membrane is considered one of the factors leading to the disruption of the BBB structure [8].

2.3. Astrocytes

Astrocytes are specialized glial cells with crucial roles in the CNS. Their end-feet cover the basal membrane on the outer surface of the BBB endothelium. They are characterized by numerous extensions containing intermediate filaments of the cell cytoskeleton and glial fibrillary acidic protein. These cells cover more than 99% of the abluminal surface of cerebral vessels [17] and contribute to the structure, development, and unique endothelial phenotype of the BBB. Their roles are thought to be facilitated by their anatomical proximity to endothelial cells and the expression and release of soluble factors [8]. The parenchymal surface of cerebral vessels is completely covered by a mosaic of astrocytic end-feet and separated by gaps of approximately 20 μm [3]. Astrocytes interacting with endothelial cells strengthen TJs and reduce the gap junction area, playing a crucial role in the limited permeability and integrity of the BBB. A study investigating the importance of astrocytes in the induction of BBB properties suggested that astrocytes interacting with endothelial cells increase transendothelial electrical resistance and that the proximity of astrocytes to the endothelium is effective in the development of a tight BBB [18]. Due to their polarized anatomical structure and the proximity of their end-feet to smooth muscle cells in arterioles and pericytes, astrocytes are also noted to play a role in regulating blood flow during neuronal activity [19]. Astrocytes contribute to various functions, including synapse formation and plasticity, energetic and redox metabolism, and synaptic homeostasis of ions and neurotransmitters like glutamate. They regulate cerebral blood flow in response to local neuronal activity changes by not only stimulating vascular smooth muscle cells but also by modulating these diverse functions [20]. Therefore, astrocytes are recognized as central to dynamic signaling within the NVU and play a significant role in coordinating neurovascular coupling [21].

2.4. Pericytes

Brain pericytes are polymorphic cells with a spherical or oval cell body and a distinct round nucleus. They also have long cytoplasmic processes with heterogeneous morphology that are positioned along the axis of the blood vessel [22], as well as secondary and tertiary processes that encircle the vascular wall with smaller, circular branches [8]. Pericytes, which contribute to the regulation of cerebral blood flow and strengthen the BBB's impermeability, are embedded in the endothelial basement membrane at varying intervals along the vessel [3]. Pericytes contain various proteins, including contractile proteins, cytoskeletal components, and surface antigens. The predominant contractile protein localized in microfilament bundles is smooth muscle actin [8]. However, their morphology and protein expression can vary along the microvascular tree [23]. Additionally, pericytes can differentiate into fibroblasts, smooth muscle cells, macrophages, and other cell populations [24]. It has also been reported that adult CNS microvascular pericytes possess neural cell potential and serve as a source of multipotential progenitor cells [25]. Significant findings indicate that pericytes can regulate blood flow in response to neuronal activity, whereas capillary diameter does not change in regions lacking pericytes. This suggests that pericytes, rather than endothelial cells, play a role in capillary diameter changes due to their possession of contractile proteins [26]. In

larger vessels, it is emphasized that myocytes are the primary effectors of changes in vessel diameter within the NVU [27]. A study investigating the *in vivo* roles of pericytes in the BBB found that pericytes regulate BBB-specific gene expression in endothelial cells, induce the polarization of astrocytic end-feet, and that the extent of pericyte coverage of capillaries is significantly related to the integrity, permeability, and regulation of the BBB [28].

Due to their subendothelial location in the microvascular structure, it has been suggested that pericytes may also play a role in regulating coagulation events that occur following cerebrovascular lesion formation [29]. It has been suggested that pericytes negatively regulate fibrinolysis in brain endothelial cells in vitro, thereby supporting procoagulant activity. Additionally, pericytes may exhibit endogenous anticoagulant activity due to their role as a major *in vitro* source of the serpin protease nexin-1, known for its antithrombin effects [30]. Therefore, pericytes, which are important components of the NVU, play an essential role in the local coagulation process due to their pro- and anti-coagulant effects.

2.5. Microglia

Microglia are a type of glial cell with immune-competent and phagocytic properties in the CNS [31], constituting approximately 10%–15% of all glial cells [32]. They are derived from myeloid progenitors in the embryonic yolk sac and proliferate to colonize the entire parenchyma after migrating to the developing neural tube [33]. They continuously monitor the brain parenchyma and play a crucial role in maintaining the homeostasis of nervous tissue [34].

Microglia are primarily characterized by two fundamental morphologies, depending on the physiological and pathological conditions in the brain. Under physiological conditions, resting microglia have slender, elongated processes that give them a branched morphology. These processes allow the cells to promptly detect homeostatic disturbances in the CNS [31]. These microglia are characterized by a small cell body (5–10 μm) and exhibit minimal or no cellular movement [35]. Their processes are constantly in motion, extending outward from the cell body to survey large areas, protruding and retracting to interact with NVU components such as neurons, astrocytes, and blood vessels. This allows them to continuously monitor the functional status of synapses [33]. A recent study has found that near the tips of the long processes of microglia, there are actin-dependent, thin, hair-like filopodia that can extend and retract more quickly than the main processes. These filopodia significantly increase the effective sensing volume of microglia [36]. During a pathological condition (such as trauma or inflammatory stimuli), microglia rapidly become activated, transforming into a phagocytic morphology characterized by a large cell body and short processes. This transformation is related to the type and severity of the damage [35] and is associated with changes in the release of cytotoxic substances such as oxygen radicals, proteases, and proinflammatory cytokines, as well as alterations in surface antigens [31]. Microglial reactivity under pathological conditions is complex, as these cells can exhibit different phenotypes over time or even simultaneously. Although the mechanism underlying these phenotype differences is not clear, reactive microglia are classified as M1 (proinflammatory and neurotoxic) and M2 (anti-inflammatory and neuroprotective) [37]. M1 microglia are notable for their proinflammatory and prokilling functions, while M2 microglia play roles in immune regulation and repair. Additionally, the phagocytic ability of M2 microglia reveals their capacity to clear cellular debris and contribute to neural repair [35]. It has been reported that activated microglia can provide immune optimization by interacting and cooperating with astrocytes, another important component of the NVU, in conditions such as neuroinflammation [38].

3. DRUG DELIVERY AND BBB OPENING APPROACHES

3.1. Challenges for drug delivery

The passage of certain substances through the BBB and their entry into the brain depends on numerous primary and secondary factors. These factors include the size of the molecule, its flexibility, conformation, ionization, charge, hydrophilic/lipophilic properties, the number of hydrogen bond donors/acceptors,

cellular enzyme secretion/stability, affinity for efflux mechanisms, and affinity for carrier mechanisms [39]. In addition, systemic enzymatic stability, affinity for plasma protein binders, uptake of the drug into non-target tissues, clearance rate, cerebral blood flow, diet, age, gender, species, and the effects of existing pathological conditions are also considered important peripheral factors [40]. It has been reported that more than 98% of large pharmaceuticals with different structures such as peptides and proteins, and even all small molecules, can not pass the BBB [41]. Theoretically, it is emphasized that for a molecule to cross the BBB in pharmacologically significant amounts, its molecular weight should be <400 Da, the molecule should be lipid soluble and should not be a substrate for an active efflux transporter at the BBB [42]. It should be also taken into account that lipophilic molecules, even if they are <400 Da, can be degraded by specific enzymes expressed by BBB cells and thus be trapped in the metabolic barrier [43].

The early stages of drug discovery studies, especially based on the design of orally applicable molecules, are guided by Lipinski's "rule of five" (Ro5), which is an experimental and computational approach related to the solubility and permeability of the molecule [44]. This rule is actually related to the four physicochemical properties of a molecule, namely its molecular weight, lipophilicity, and the number of hydrogen bond donors or acceptors, and is named after the cutoff points of each parameter being close to 5 or a multiple of 5. According to this rule, it is reported that poor absorption or permeability, i.e. less oral bioavailability, is more likely in the following cases: i) molecular weight >500, ii) calculated Log P (CLogP) value is above 5 (partition coefficient, P, value is parallel to the hydrophobicity of the molecule), iii) more than 5 hydrogen bond donors, and iv) more than 10 hydrogen bond acceptors [45]. Today, the Ro5 rule is still used in brain studies which are based on drug design, synthesis, and oral bioavailability [46].

For assessing BBB passage, the concentrations of therapeutic drugs in the blood and pharmacokinetic calculations need to be considered. These calculations utilize the equation [%ID/g = PS × AUC] [43,12]. In this equation, "%ID/g" is the percent injected dose per gram of brain, "PS" is the brain permeability surface area, and "AUC" is the steady-state area under the plasma concentration curve [47]. Accordingly, it is noted that drug uptake by the brain can be increased through stabilizing the drug in the blood (e.g., increasing AUC) and making modifications to the drug structure to enhance passive permeability and/or specificity via transport systems. Strategies such as drug manipulation and opening/circumventing the BBB have been emphasized for this purpose [12]. Similarly, it has been suggested that transiently increasing BBB permeability in a controlled and safe manner through various methods could enable higher drug concentrations to reach brain tissue from the bloodstream, potentially offering hope for treating localized malignant diseases in the brain [48].

3.2. Invasive and non-invasive strategies

Various mechanisms are involved in the passage of substances through the BBB. These mechanisms include paracellular diffusion, transcellular diffusion, carrier-mediated transport, receptor-mediated transport, absorptive-mediated transport, and cell-mediated transport. Additionally, the efficacy of efflux transporters in removing harmful substances that reach the CNS is also emphasized [49]. Mechanical damage (trauma, surgical operations), ischemia (reduced/blocked blood flow), infiltration of immune cells (inflammation), activation of matrix metalloproteinases, increase in reactive oxygen species (oxidative stress), chemicals (exposure to heavy metals, pesticides), radiation, and some biological factors can lead to disruption of BBB integrity [50]. It is known that some primary brain tumors also damage the BBB and increase its permeability; however, this is a limited increase, and these malignancies are protected from therapeutic agents due to the intact blood–tumor barrier in peritumoral regions [51]. For these reasons, there is an important need to develop new drugs to cope with various pathologies affecting the central nervous system, and various strategies are being followed to overcome the problem of these drugs not being able to cross the BBB. In these studies, various invasive and noninvasive methods have been employed. Invasive methods include direct injection of drugs into the brain parenchyma for therapeutic purposes,

manipulation of certain hyperosmolar solutions [52], implantation of controlled release systems [53], and focused ultrasound using microbubbles [54]. Noninvasive methods include the nose-to-brain pathway, inhibition of efflux transporters, chemical modification of drugs, and the use of nanocarriers (such as liposomes, lipid/polymeric/inorganic nanoparticles, nanogels, and nanoemulsions) [49]. The intracranial drug delivery, one of the invasive methods, has been considered as an interesting treatment strategy compared to systemic application in difficult-to-treat epilepsies. Although this method has some advantages, such as giving the opportunity to use substances that are impermeable to the BBB, reducing the risk of systemic/neurological side effects, and reaching high drug concentrations in the targeted localization, and some disadvantages such as being an invasive procedure and difficulty in long-term administration of the drug have been reported [55]. The nose-to-brain route, one of the non-invasive methods, not only increases the brain bioavailability of the drug by reaching the central nervous system directly through the olfactory and trigeminal nerve pathways, but also it is a promising route even in aggressive brain cancer such as glioblastoma due to the high vascularization of the nasal mucosa, reduced systemic metabolism, low risk of infection and suitability for chronic use [56,57]. The safety, efficacy, feasibility, advantages, disadvantages, and potential complications of these approaches in clinical applications have been debated for years and continue to be a topic of ongoing discussion.

4. CONCLUSION

The BBB maintains the integrity and homeostasis of brain cells under physiological conditions by providing a physical barrier, a metabolic barrier, and selective transport systems. It protects the brain from changes in the blood, metabolizes and modifies substances in the blood and brain, and ensures an optimal environment for neurons. However, while this vital system protects the brain from potentially harmful compounds, it also presents a major barrier to the passage of therapeutic drugs into the brain parenchyma in various brain pathologies. It appears that various physicochemical properties of substances used for therapeutic purposes and some peripheral factors significantly affect BBB bioavailability. Various invasive or non-invasive strategies are being followed in order to increase the effectiveness of treatment in various brain diseases by overcoming the BBB barrier in a controlled manner. In recent years, some of the non-invasive strategies are promising even in the most aggressive brain cancers. Considering the wide spectrum of central nervous system diseases, it is clear that there is a significant need for new drug designs or formulations with pharmacokinetic profiling and the development of optimal treatment strategies.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, B.C. and İ.Ö.A; Writing, B.C.; Supervision, İ.Ö.A. All authors have read and agreed to the published version of the manuscript.

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