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## Mechanisms of dysfunction of the blood-brain barrier in critically ill patients: emphasis on the role of matrix metalloproteinases

*Mecanismos de disfunção da barreira hematoencefálica no paciente criticamente enfermo: ênfase no papel das metaloproteínas de matriz*

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### ABSTRACT

This paper aims to describe the physiological basis of the blood-brain barrier components and its properties. Additionally, the particular effects of metalloproteinases and their control over the extracellular matrix and its relationship with blood-brain

barrier dysfunction are discussed. Finally, the role of metalloproteinases on changes in the central nervous system in critically ill patients is discussed.

**Keywords:** Blood-brain barrier; Extracellular matrix protein; Matrix metalloproteinases

### INTRODUCTION

The concept of the blood-brain barrier (BBB) was initially presented at the end of the XIX century in Germany, based on Paul Ehrlich's studies where after injecting dyes into the arterial and venous circulation of animals, he observed that all organs except the spinal cord and brain were colored, leading him to hypothesize the existence of two compartments. In addition, in a review by Hugh Davson,<sup>(1)</sup> the studies of Bield and Kraus in 1989 and Lewandowsky in 1900 are mentioned, in which the lack of pharmacologic effects on the central nervous system (CNS) of systemically administered substances was ascribed to this barrier. Years later, in 1913, Edwin Goldmann (one of Ehrlich's students) observed the opposite phenomenon when he injected a dye directly into the cerebrospinal fluid of animals; this dye stained the entire CNS but not any peripheral organ.<sup>(2)</sup> In the 1960s, Reese and Karnovsky and Brightman and Reese repeated Ehrlich's and Goldmann's experiments on a structural level using electronic microscopy and identified CNS capillary vessels and endothelial cells as the site of the blood-brain barrier.<sup>(1)</sup>

### Blood-brain barrier (BBB)

#### BBB description

The central nervous system (CNS) is an area protected by three structural elements: the BBB, an interface between the brain and blood vessels; the blood-cerebrospinal fluid barrier (BCSFB), formed by the choroid plexus and arachnoid membrane along with blood vessels and cerebrospinal fluid; and the blood-arachnoid barrier (BAB), which is the interface between blood vessels and the arachnoid epithelium underlying the meningeal dura

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mater.<sup>(3)</sup> These barriers are specialized CNS structures regulating brain, spinal cord and cerebrospinal fluid homeostasis in relation with the remaining systems of the body.<sup>(4,5)</sup>

## Components of the BBB

### - Endothelial cells

The barrier is formed by endothelial junctions that control the coordinated opening and closing of cell-cell junctions.<sup>(6)</sup> The endothelial junctions are made up of different multi-protein complexes, including tight junctions (TJs) and adherens endothelial junctions (AEJs), which are the main cell permeability regulators.<sup>(7)</sup> TJs consist of three integral membrane proteins, claudin, occludin and junctional adhesion proteins, and a number of accessory cytoplasmic proteins, including zonula occludens (ZO) proteins 1, 2 and 3, cingulin, and other membrane-associated guanylate kinase (MAGUK) proteins. These accessory proteins connect membrane proteins to actin to maintain the integrity of endothelial structure and function. AEJs are made up of membrane proteins called cadherins that are connected to actin via intermediate proteins called catenins ( $\alpha, \beta, \gamma$ ) to form adhesive intracellular contacts and interact with TJs. Brain endothelial cells (BECs) lie over a basal lamina containing extracellular matrix molecules, covering more than 90% of the surface of the endothelial cells, and are involved in BBB permeability.<sup>(8,9)</sup>

### - Occludins

Occludins were the first membrane proteins to be identified in endothelial cells TJs. Occludin is a 65 kDa phosphoprotein with four transmembrane domains and is connected to two extracellular tyrosine-rich moieties in the intracellular carboxy and terminal amino domains. The function of occludin should be mostly viewed in a regulatory context rather than as a relevant structural protein to establish the properties of the barrier. Its carboxy terminal cytoplasmic domain provides the connection between occludin and the cytoskeleton, providing high electrical resistance of endothelial cell monolayers and reducing the paracellular permeability.<sup>(10)</sup>

### - Claudins

Claudins are 21-28 kDa integral proteins with four transmembrane domains, two extracellular loops and carboxy and amino terminal cytoplasmic domains with

a short intracellular loop.<sup>(11,12)</sup> Extracellular moieties are bound to claudins of adjacent endothelial cells, forming the primary TJ fence.<sup>(11)</sup> The main role of claudins is the regulation of paracellular selectivity to small ions. To regulate this paracellular selectivity, the interactions between different classes occur in two distinct ways: laterally on the membrane level (heteromeric interactions) or head-to-head for adjacent cells (heterotypical interactions).<sup>(13,14)</sup>

### - Astrocytes

In the human brain, astrocytes are star-shaped glial cells that are usually ten times more abundant than neurons,<sup>(15)</sup> and the extensions of astrocytes form a close and adhered lamellar net on the external BBB endothelial surface and respective basal membrane.<sup>(16)</sup> Increasingly, in vitro and in vivo data support the hypothesis that astrocytes are excitable cells and play a relevant role in information processing and neuron activity modulation.<sup>(17)</sup> These cells are important for vascular tonus control;<sup>(15)</sup> therefore, the role of astrocytes in the maintenance of brain microvasculature endothelial cells and in signaling has been studied in individuals with different diseases and in studies of BBB integrity.<sup>(18)</sup>

### - Pericytes

Pericyte recruitment and endothelium interaction are essential for BBB formation, maturation and maintenance. Pericytes communicate directly with endothelial cells via invaginations called 'peg sockets', where one single pericyte may contact several endothelial cells, providing an additional communication layer<sup>(19)</sup> and mechanical stability for the vessels. Pericytes were shown in recent trials to be contractile brain cells that regulate the capillary blood flow, playing a relevant role in the maintenance of the BBB, along with brain homeostasis self-regulation.

In vivo studies have determined that the interactions of pericytes with endothelial cells are critical for BBB regulation, and disruption of these interactions may lead to barrier dysfunction and neuroinflammation.<sup>(20)</sup> Additionally, in vitro studies have shown that pericytes express molecules such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) that regulate the BBB integrity.<sup>(21)</sup>

### - Extracellular matrix (ECM)

The extracellular space represents about 20% of the total brain volume and is filled with the highly

organized extracellular matrix (ECM), which is located at the interface of the blood vessels and the glial cells. The ECM apparently acts as an endothelial anchor by interacting with integrin-type endothelial receptors,<sup>(7)</sup> which play a fundamental role in cell signaling, cell migration and brain capillary formation during angiogenesis.<sup>(9)</sup> These cell-matrix interactions may stimulate a series of intracellular signaling pathways in vascular cells, neurons and support glial cells and may additionally be essential to the BBB because they are likely to have a role in the maintenance of TJ endothelial proteins.<sup>(7,16,22)</sup>

ECM rupture may lead to changes in the cytoskeleton of brain microvasculature endothelial cells that in turn affect TJ proteins and barrier integrity, resulting in increased permeability in disease conditions.<sup>(16)</sup>

### Blood-brain barrier functions

The BBB is an important part of the communication network connecting the CNS and peripheral tissues and additionally works as an interface limiting and regulating substance exchange between the blood and the CNS.<sup>(23)</sup>

BBB impermeability results from several unique features that make difficult for molecules trying to cross this barrier. This property is based on the extremely restrictive endothelial permeability and the high concentration of degrading enzymes within the endothelium. Because of this very limited permeability, except for water, gases such as oxygen and carbon dioxide and some very small liposoluble molecules can cross the barrier intact.<sup>(23)</sup> Hydrophilic molecules that are essential for brain metabolism, such as ions, glucose, amino acids and nucleic acid components, cross the BBB via specialized channels.<sup>(24)</sup> The transport of hydrophilic molecules, such as peptides and proteins that have no specific transport system, is much slower than for lipophilic molecules; however, the amounts crossing the BBB may be enough to cause a neuron-receptor-mediated effect. Some special proteins and peptides, such as peripheral hormones and regulatory brain-acting peptides, usually have their own specialized saturable systems all over the BBB. Therefore, the BBB is highly restrictive but is still unable to prevent some toxins and therapeutic agents from crossing from the blood stream into the brain.<sup>(24,25)</sup>

In addition to selective permeability, the BBB possesses relevant properties such as neuroimmune functions, including the secretion of cytokines,

prostaglandins and nitric oxide. The BBB can be stimulated by one compartment (e.g., the systemic) and simultaneously respond with secretions to the other (e.g., the central nervous system); this function is central for the neuroimmune response. For example, LPS applied to the abluminal surface of endothelial brain cells stimulates IL-6 secretion from its luminal surface.<sup>(25)</sup>

### Matrix metalloproteinases (MMP)

Extracellular matrix metalloproteinases are enzymes normally found in their inactive form. These proteins are part of the zinc-dependent proteinases family, with 23 members identified in humans to date.<sup>(26)</sup> These proteins are categorized as follows: a) true collagenases (MMP: 1, 8, 13), which digest triple-helix collagen; b) gelatinases (MMP: 2, 9), which act on collagen and denatured gelatin; c) stromelysins (MMP: 3, 10, 11), which degrade proteoglycans; d) matrilysins (MMP: 7, 26), which degrade proteoglycans, fibronectin and laminin; e) membrane-bound metalloproteinases or MT-MMPs (MMP: 14, 15, 16, 17, 23, 24, 25), in addition to other enzymes that catalyze the gelatin degradation of fibronectin, aggrecan and other ECM substrates; and f) other MMPs (MMP: 12, 19, 20, 21, 27 and 28),<sup>(27)</sup> which are able to degrade all EMC components and connective tissue proteins.<sup>(28)</sup>

MMP effects are strictly controlled by three mechanisms: (1) gene transcription regulation; (2) pro-enzymatic activation regulation; and (3) inhibition by specific molecules (TIMP).<sup>(27)</sup> MMP gene expression is controlled in response to cytokine (IL1, 4, 6 and TNF $\alpha$ ) stimulation, reactive oxygen species (ROS), nitric oxide (NO), and hormones and growth factors that binding to cell surface receptors, triggering intracellular signaling cascades that, in turn, activate transcription factors that are bound to responsive regions on different MMP promotion genes.<sup>(29)</sup> MMPs are normally found inactive in the cytosol and may be cleaved by proteases such as plasmin, tissue plasminogen activator (tPA), or other MMPs into their active forms.<sup>(30)</sup> Endogenous tissue inhibitors of metalloproteinases (TIMP) are the main physiological MMP inhibitors; additionally, serum  $\alpha$ -2 macroglobulin can inhibit the activity of MMPs. TIMPs are a group of proteins that are able to inhibit soluble MMPs in stoichiometric 1:1 ratios, forming TIMP-MMP complexes that render the protease unable to bind to the substrate. Currently, four TIMP family members are known, and MMPs may be inhibited by a

number of different TIMPs.<sup>(30)</sup> Each TIMP has unique features: TIMP-1, TIMP-2 and TIMP-4 are in their soluble form, and TIMP-3 is strongly connected to the ECM<sup>(31)</sup>. These inhibitors are related to cell growth promotion, synaptic plasticity and antiangiogenic and antiapoptotic activities.<sup>(32)</sup>

### Blood-brain barrier dysfunction

BBB dysfunction, sometimes called 'BBB opening,' has been described to be central to CNS disease progression.<sup>(33)</sup> This dysfunction is probably related to exposure of the cerebral microenvironment to partially noxious substances, which may result in the loss of homeostasis, neuronal signaling or impairment and cell death.

The central role of MMPs in neurovascular matrix degradation and the genesis of different disease states have been shown in several neuroinflammation models. The activation and expression regulation of MMPs is complex and well controlled, and the loss of this control is involved in the BBB-disrupting pathophysiologies. After BBB disruption, neurotoxic substances are allowed to circulate into the brain parenchyma, with leukocyte infiltration and microglia activation, promoting the inflammatory response found in critically ill patients with different diseases, such as stroke and head trauma (HT).<sup>(16,34)</sup>

Studies in stroke animal models have demonstrated MMP activation, leading to basal lamina and ECM degradation with BBB disruption. This disruption leads to the increased fragility of small vessels, contributing to the transition of an initially ischemic stroke into a hemorrhagic stroke. In addition, MMP-2 activation was observed in transient ischemic attack in the first steps of injury with consequent claudin degradation (part of the TJ structure, resulting in BBB disruption). MMP-9 is also apparently relevant in the acute phase in peri-infarction areas because it is related to neutrophil infiltration around capillary vessels, with worsened stroke-associated neuron death.<sup>(30,34)</sup> Although most investigations have focused on the functions of MMP-2 and MMP-9, other family members may also play relevant roles. For instance, MMP-3 may be activated post-brain ischemia/reperfusion in rats, causing ECM disruption and contributing to BBB opening, leading to neuroinflammation. Finally, MMP-13 was recently found in the brain of post-ischemic rats and was found in the nuclei of post-ischemic neuron cells and in hippocampal vessels, related to BBB disruption in stroke.<sup>(35,36)</sup> These changes could also be relevant to thrombolytic-

associated brain bleeding. These therapeutic agents are associated with MMP activation, and, in animal models, pharmacologic MMP inhibition was able to reduce tPA-associated brain hemorrhage volumes.<sup>(37,38)</sup>

Post-mortem human studies have confirmed that MMP-9 is increased in the brain tissue in infarction and peri-infarction zones of stroke patients.<sup>(39)</sup> In a recent study, also in humans, an association between plasma MMP-9 levels and BBB disruption was found following stroke.<sup>(40)</sup> Together, cumulative experimental and clinical data suggest that MMPs may be a biomarker of neurovascular injury during the acute phase of stroke and suggest the use of tPA in conjunction with MMPs in patients eligible for thrombolysis.<sup>(37-41)</sup>

In animal HT model studies, increased MMP-9 levels were shown in cortical and hippocampal regions. MMP-2 was increased in this model days after the HT, suggesting an MMP activation cascade where fast MMP-9 activation<sup>(42)</sup> may be involved in sustained MMP-2 activation.<sup>(43)</sup> These changes are both temporally and spatially associated with BBB disruption and cerebral edema formation, and the use of MMP inhibitors leads to a reduced damage area and brain water leakage. In an HT model, MMP-9 transgenic mice showed significantly smaller injuries compared to wild-type mice. In addition to MMP-2 and -9, MMP-3 was also observed in an animal HT model, leading to vasogenic edema and consequent cell death.<sup>(44)</sup> Human studies found increased MMP-2 and MMP-9 levels in the plasma and brain during early the HT phase,<sup>(45)</sup> in addition to a close relationship between cytokines and increased acute-phase MMPs.<sup>(36,41,46-50)</sup>

In animal studies on status epilepticus induction, a hippocampal MMP-2 increase was found.<sup>(51,52)</sup> In the sera from children with status epilepticus, MMP-9 levels were found to be significantly higher and the levels of TIMP-1 inhibitors significantly lower when compared to the control patients, which could induce BBB dysfunction and brain edema.<sup>(53)</sup>

### CLOSING REMARKS

Molecular neurobiology advances are quickly providing a better understanding of acute CNS changes in critically ill patients with different conditions. Our challenge is to precisely determine which MMP changes in critically ill patients are relevant for CNS dysfunction and how therapeutic interventions targeting these enzymes could contribute to BBB stabilization to mitigate neural dysfunctions.

## RESUMO

Será descrito a base fisiológica dos componentes da barreira hematoencefálica e suas propriedades. Além disto, pretende-se abordar o efeito particular das metaloproteinases e seu controle sobre as propriedades da matriz extracelular e a relação disto com disfunção

da barreira hematoencefálica. Finalmente se demonstrará o papel da metaloproteinases nas alterações do sistema nervoso central em doenças associadas ao paciente criticamente enfermo.

**Descritores:** Barreira hematoencefálica; Proteína da matriz extracelular; Metaloproteinases da matriz

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