BLOOD-BRAIN BARRIER DISRUPTION DURING ACUTE ISCHEMIC STROKE - THE ROLE OF MATRIX METALLOPROTEINASES AND TIGHT JUNCTIONS PROTEINS

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Abstract Ischemic stroke is a major cause of death and disability worldwide. Intravenous administration of recombinant tissue plasminogen activator (rtPA) is the only approved pharmacological therapy for patients with acute ischemic stroke. Treatment with intravenous rtPA is associated with increased rates of intracranial hemorrhage. This review discusses recent findings about the changes of the components of neurovascular unit and the alteration of blood-brain barrier during stroke. The analysis provide information in order to identify factors with predictive value for hemorrhagic transformation of ischemic stroke both within or after the thrombolysis time window.

Key words: blood-brain barrier, ischemic stroke, hemorrhagic transformation, neurovascular unit, tight junctions matrix metalloproteinases, occludin, claudin, aquaporin 4

Introduction

Ischemic stroke is a major cause of death and disability worldwide. Every year ischemic stroke kills 2.9 million people and leads to 3.4 million years lived disability. One of the most important complications of ischemic stroke is hemorrhagic transformation, occurring in 30-40% of clinical cases. This complication occurs early in the course of stroke progression, within 48-72 hours of stroke onset [1-3]. The main causes of hemorrhagic transformation of ischemic stroke are the loss of microvascular integrity and disruption of blood-brain barrier (BBB) / neurovascular unit (NVU) homeostasis.

Intravenous administration of rtPA is the only Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved pharmacological therapy for treatment of patients with acute ischemic stroke [4]. Its use is associated with improved outcomes for patients who can be treated within 3 hours of the last known well time before symptom onset and a mildly more selective spectrum of patients who can be treated between 3 and 4.5 hours of the last known well time [5]. Most importantly, earlier treatment is more likely to result in a favorable outcome. Patients within 3 hours of onset with major strokes (NIHSS score >22) have a very poor prognosis, but some positive treatment effect with intravenous rtPA remains [6].

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Treatment with intravenous rtPA is associated with increased rates of intracranial hemorrhage, which may be fatal. 100 patients need to be treated with rtPA for one significant adverse outcome to occur [7,8].

Analyzing the components of NVU and the alteration of BBB during stroke might provide important information in order to identify factors with predictive value for hemorrhagic transformation of ischemic stroke both within or after the thrombolysis time window.

The blood-brain barrier / neurovascular unit structural components

The blood-brain barrier (BBB) is a dynamic interface between the peripheral circulation and the central nervous system (CNS). The anatomical substrate of BBB is the cerebral microvascular endothelium which together with the closely associated astrocytes, pericytes, neurons, other supporting cell (microglia and oligodendroglia) and extracellular matrix (ECM) constitute a "neurovascular unit" (NVU) [9]. This provides a framework for a bi-directional communication between neurons and their supply microvessels with the participation of astrocytes.

Microvessel-neuron relationship differs into the white and grey matter of the brain [10]. During cerebral development, microvessels and neurons are arranged relative to one another by growth along extracellular matrix paths [11,12]. Astrocytes and endothelial cells interact to form the basal lamina barrier and the tight junctions (TJs) responsible for permeability barrier of capillaries [13-18]. In the cerebral grey matter, the arterial supply is arranged in a series of hexagonal stacked arrays descending from the pial supply to the dependent capillary beds [19,20. forming a hierarchical organization similar to a honeycomb. Within the striatum the neuron-microvessel distribution is more homogenous, the capillary arrangement has branch points at approximately 30 μm intervals [21]. Within the white matter, capillaries are arranged in line with axons and comprise approximately 10% of the capillary density found within the grey matter [22]. This arrangement of the vasculature corresponds to the differences in regional cerebral blood flow (rCBF): the flow is lowest in the striatum and highest in the cortical grey matter. The modulation of cerebral blood flow depends on microvessels response to neuronal activation, requiring the function of intact neurons.

The microcapillary endothelium comprises tight junctions (TJs) and adherens junctions (AJs) that have the role to restrict permeability across endothelium.

Tight junctions

The tight junctions (TJs) are continuous membrane strands located on the apical site between brain endothelial cells (ECs), which consist of transmembrane proteins (junctional adhesion molecule-1, claudins and occludin) and cytoplasmic proteins (zonula occludens 1 to 3) linked to the actin cytoskeleton [23]. Among the claudin (CLN) family members, claudin 5 has been shown to be a major cell adhesion molecule of BBB TJs [24]. Occludin (OCLN) is also a tight junction strand component and contributes to junction properties and regulates permeability [25]. The structure and function of TJ proteins can be regulated by altering their expression and/or distribution at the BBB during ischemic stroke. Phosphorylation is a major regulatory mechanism of both transmembrane and accessory proteins at the TJs [26-28].

Adherens junctions (AJs) mediates the adhesion of ECs to each other and are ubiquitous in the vasculature. AJs are composed mainly of vascular endothelial VE-cadherin, a Ca2+ - regulated protein that facilitates cell to cell adhesion by forming continuous belt near the apical end of the junctional cleft [29]. AJs are involved in the localization and stabilization of the TJs. VE-cadherin upregulates TJ protein claudin-5, suggesting a direct regulation of TJ integrity by AJ proteins [30].

Matrix metalloproteinases (MMPs)

MMPs, also called matrixins, comprise a family of enzymes that cleave protein substrates by using a mechanism involving activation of a site-bound water molecule by a Zn2+ ion. MMPs consist of 23 proteases in humans, classified according to their substrate specificity in four classes: the collagenases (MMP-1, -8 and -13), the gelatinases (MMP-2 and -9), the stromelysins (MMP-3, -10 and -11) and a heterogeneous group containing matrilysins (MMP-7), metallo-elastase (MMP-12), enamelysin (MMP-20), endometase (MMP-26) and epilysin (MMP-28) [31]. Most MMPs are synthesized as inactive latent enzymes. Conversion to the active enzyme is generally mediated by activator systems that include
plasminogen activator or pro-hormone convertase, furin. Although the catalytic domain of MMPs isstructurally similar, there are many differences in substrate specificity, cellular and tissue localization, membrane binding and regulation, making this a versatile family of enzymes with a multitude of physiological functions, which are not fully understood. Essentially, all members of the MMP family have been linked to disease development, notably to cancer metastasis, chronic inflammation with ensuing tissue damage, as well as to neurological disorder [32]. MMPs activity is regulated by a group of endogenous proteins called tissue inhibitor of metalloproteinases (TIMPs), which bind to active and alternative sites of activated MMPs [33]. Until now there were identified four homologous TIMPs (TIMPS 1-4), proteins with size 21-30 kDa [34]. The injured brain has various cell types that can express MMPs, including resident cells and infiltrating inflammatory cells. The specific MMPs expression differs with brain region, cellular sources and type, severity and duration of injuries [35]. MMPs can also be released from invading leukocytes [36]. For example MMP-8 is known as the 'neutrophil collagenase' [37].

Role of MMPs in BBB disruption

BBB opening correlates with redistribution of the TJ and AJ proteins from the plasma membrane to the cytoplasm as well as reorganization of the endothelial actin cytoskeleton. The extent of BBB disruption is related to type, severity and duration of ischemic insult. The expression of MMPs in adult brain is very low in normal circumstances but during ischemic stroke several MMPs are upregulated and activated [38,39]. MMPs disrupt the basal lamina proteins, leading to BBB leakage, leukocyte infiltration, brain edema and hemorrhage. Evidence suggests that MMP-2 and MMP-9 play different roles in BBB disruption during ischemic stroke. MMP-2 knock-out does not provide neuroprotection in mouse models of permanent and transient middle cerebral artery occlusion [40]. Also in vitro studies showed that MMP-2 is not toxic to neurons in hippocampal slice preparations [41]. In contrast MMP-9 knock-out provides strong neuroprotection in the same animal models and in vitro MMP-9 is toxic for neurons in hippocampal slice preparations and in cultured cortical neurons. These data were supported by the results of a clinical study. Lucivero and colab. [42] reported an increase in plasma MMP-2 only in patients with lacunar (mild) ischemic stroke, early (within 12h) and related to a better outcome. In contrast, an increase in plasma MMP-9 was observed later (at day 7) and related to more severe stroke. MMPs are thought to have beneficial roles in stroke recovery. Following injury, blood vessels are dependent on plasminogen activator system and MMPs for their regeneration [43]. A balanced level of MMP activity is important for vascular remodeling after ischemic stroke [44]. Therefore an extended inhibition of MMPs through the use of broad spectrum inhibitors might prove deleterious [45,46].

Aquaporin-4 dysfunction during BBB disruption

Aquaporin-4 dysfunction during BBB disruption is also involved in the development of cytotoxic and vasogenic edema [47]. Aquaporins (AQP) are highly permeable water channels widely found in different tissues of the body [48]. In the central nervous system (CNS) are expressed two AQP: AQP1 and AQP4. AQP4 expression is restricted to astrocytes throughout the brain and spinal cord and the ependymal cells that line the cerebral ventricles [49]. Several studies using animal models of ischemic stroke yielded contradictory results, showing up-regulation or down-regulation of AQP4 following acute ischemic stroke, depending mostly on the duration of vessel occlusion. The demonstration of rapid but selective loss of astrocytic AQP4 suggests that brain astrocytes may possess the cellular machinery for a self-protective response to early ischemia; however, the localization of this effect to regions that are supplied by blood vessels that have been severely damaged by stroke may indicate that there is an injury threshold for this effect [50].

The mechanisms of BBB disruption during ischemic stroke

Loss of regional cerebral blood flow (rCBF) and increased vascular resistance due to mechanical obstruction of vessels by a thrombus or emboli results in loss of oxygen and nutrients to the surrounding tissue. The endothelium in the core ischemic zone sustains the greatest degree of insult, but measurable changes in blood brain barrier tight junctions (BBB TJ) permeability require hours of continuous reduction of blood-flow [51]. TJs within the core zone have a different time-frame response in comparison with endothelium of the surrounding tissue (penumbra zone). Therefore the components of NVU undergo different molecular/cellular events, phasic response and degrees of TJs...
permeability after an ischemic event. Throughout the infarcted region starts a cascade of events: depletion of ATP, excitotoxic glutamate efflux (neuronal component), ionic imbalance (increased intracellular calcium), loss of metabolic function with increased acidosis, oxidative stress and activation of inflammatory processes. Lactacidosis contributes to swelling of endothelial cells, neurons and astrocytes and as a consequence the capillary vessels diameter shrinks. This is the phase of cytotoxic edema. There is an induction of protease (tissue plasminogen activator (tPA), matrix metallo-protease (MMPs), cathepsins and heparanases that contribute to BBB extracellular matrix (ECM) degradation. These enzymes may perpetuate BBB permeability through integrin mediated mechanisms [52,53]. Within minutes of the occlusion there is an increased expression of early response genes (c-fun, c-fos), followed later (hours) by an increase in heat shock genes (eg. Hsp70, HSP72) [54,55]. Within the penumbra apoptotic pathways are induced through both caspase dependent (ATP-dependent) and caspase independent mechanisms [56,57]. There is also a disorder of TJ protein activity during ischemic period. In animal models of stroke with reperfusion, altered distribution or loss of TJ proteins is frequently seen in ischemic cerebral microvessels, resulting in compromised BBB integrity [58]. In the same studies MMP-2 and MMP-9 have been sown to mediate the degradation of several TJ proteins including occludin, claudin-5 and zonula occludens-1. Reperfusion due to reestablishment of CBF to the ischemic zone may cause hemorrhagic transformation. There is an initial reperfusion permeability (duration of minutes) associated with acute elevation in rCBF, which is followed by a 'biphasic' permeability response [59]. Reactive hyperemia and loss of cerebral autoregulation upon initial reperfusion account for the acute opening of the BBB and disassembly of TJs. This acute phase is passively dependent on perfusion and is often concurrent with a sharp increase in blood pressure [60]. The TJ reassembly encompass endothelial, pericyte and ECM interactions, accompanied by reestablishment of autoregulatory responses. Cerebral pericytes containing contractile proteins, have the potential to regulate CBF and the ECM containing collagen act to support and anchor the capillary endothelium. Following the initial hyperemia, hypoperfusion of ischemic area occurs (no-reflow effect), resulting in deficiency of nutritional support for a sustainable recovery of the tissue. This hypoperfusion is attributed to multiple factors: continued cerebral metabolic depression, microvascular obstruction, occlusion via endothelial and astrocytic end-feet swelling and the formation of endothelial microvilli [61]. Hypoperfusion may enhance neutrophil adhesion and inflammation. This first phase of the biphasic permeability, with duration of 3-8 hours after reperfusion, has been attributed to increased inflammatory and oxidative stress on the BBB, in conjunction with enzymatic degradation (MMP-9) of the ECM [62,63]. The no-reflow effect appears more significant with extended periods of ischemia or if the ischemia is associated with venous obstruction. Increasing duration of ischemia potentiate the final phase of the biphasic BBB TJs permeability. The second phase of biphasic permeability coincide with angiogenesis and increased vasogenic edema and appear at 18-89 hours after reperfusion, dependent on ischemic severity and brain region evaluated. However neurovascular remodeling may continue weeks after the ischemic event [64,65]. The inflammatory activity contributes to this final phase and TJs undergo a process of disassembly and reassembly.

**Concluding remarks**

Blood-brain barrier (BBB) dysfunction is an important pathophysiological process in ischemic stroke and occurs immediately after occlusion of the vessel. This early ischemic BBB damage during thrombolytic window is closely associated with hemorrhagic transformation and thus the extensive scientific interest in understanding this process in order to develop therapies to target it and reduce hemorrhagic complications of thrombolytic stroke therapy. Understanding the dynamics and the role of MMPs after ischemic stroke will have important implications in development of therapies aimed to modulate MMPs. Analysis of the circulating TJ protein levels may enable a screening of patients with high risk of hemorrhagic transformation after thrombolytic
therapy and may also constitute a biomarker for identification of patients with ischemic stroke outside the therapeutic window that may still benefit from thrombolytic therapy. However, in view of the complex pathophysiology of ischemic stroke, further studies are needed in order to translate the experimental findings into clinical practice.

**Conflict of interest**

Disclosure of conflict of interest statement: none.

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