Endothelial Cells and Blood-Brain Barrier (BBB): The Key Factors in Ineffective Reperfusion Outcomes

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Abstract

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Abstract

In the realm of neurointerventional treatment after ischemic stroke, ineffective reperfusion represents a significant challenge, with the integrity of the blood-brain barrier (BBB) serving as a pivotal determinant of outcomes. This review sheds light on the unique characteristics and roles of brain endothelial cells within the context of stroke with ineffective reperfusion. We address the distinctiveness of brain endothelial cells relative to their counterparts in different tissues, outlining their pathophysiological transformations, functional impairments, and inflammatory cascades post-stroke. The differential gene expression between brain endothelial cells and those from other organs provide a deeper understanding of their intrinsic roles in neuroprotective therapy. Looking ahead, exploring analogies between brain endothelial cells and those from organs with similar ischemia-reperfusion injury profiles could lead to innovative therapeutic strategies. This review highlights the paramount importance of understanding the nuanced roles of endothelial cells in mediating BBB dynamics, ultimately influencing reperfusion outcomes.

Keywords: Stroke, Neurovascular Units (NVU), Tight Junctions, Inflammation, Pathological Mechanism

Introduction

Acute ischemic stroke stands out as a remarkable health challenge, consistently asserting itself as a principal cause of mortality and long-term disability worldwide. Endovascular Treatment (EVT), with its targeted recanalization approach, has evolved as an invaluable tool for treating acute ischemic strokes arising from occlusions in large blood vessels. The application of EVT has led to significant reductions in stroke-related mortalities, while also amplifying the prospects of enhanced recovery and improved prognoses for patients [1,2]. However, the clinical setting of stroke management reveals a more complex narrative. Successfully achieving thrombus clearance within the stipulated treatment window, though crucial, did not consistently translate to positive patient outcomes. A subset of patients, despite the timely and successful removal of occlusions, experience an irritating deterioration in neurological functions. An insightful observation from the Highly Effective Reperfusion Evaluated in Multiple Endovascular Stroke Trials (HERMES) serves to accentuate this conundrum [3]. Although 71% of the cohort achieved successful vascular re-opening, only 46% of the successful cases noted positive outcomes.

These clinical situations, where patients presumably benefit from successful reperfusion subsequently manifest suboptimal clinical results, fall under the ambit of ineffective reperfusion [4]. This phenomenon highlights a crucial knowledge gap in our understanding of post-stroke recovery dynamics. Consequently, this review seeks to lay out the complexities of the Blood-Brain Barrier (BBB) and endothelial cells, as examining their potential roles in these perplexing outcomes with ineffective reperfusion after EVT. As primary gatekeepers of cerebral homeostasis and vascular function, the BBB and endothelial cells (ECs) may well hold the key to revealing the mysteries of ineffective reperfusion, guiding future therapeutic strategies, and patient management paradigms.

Blood-Brain Barrier, Neurovascular Units and Endothelial Cells

BBB refers to the unique structure of the microvascular system within the central nervous system (CNS). Unlike many other blood vessels, CNS vessels are continuous nonfenestrated vessels, and possess distinctive properties that facilitate strict regulation over the passage of molecules, ions, and cells between the blood and brain [5-7]. This stringent regulation enables BBB to meticulously maintain CNS homeostasis, which is vital for its proper function. It also protects the CNS from harmful entities like toxins, pathogens, inflammation, and from potential injuries and diseases. Presence of neurological conditions, including stroke, multiple sclerosis (MS), brain trauma, and neurodegenerative diseases, a compromised BBB often plays a crucial role in the pathology and progression of diseases [5-7]. Impaired BBB function can lead to ion imbalances, disrupted signaling homeostasis, and the unwarranted entry of immune cells and inflammatory agents into the CNS, culminating in neuronal dysfunction and degeneration.

Endothelial cells, in conjunction with perivascular cells (PC), basement membrane, astrocytes (ACs), microglia (MGs), and nerve endings, constitute the neurovascular units (NVU)(Figure 1). The NVU, foundation of BBB, holds pivotal roles such as maintaining local homeostasis, supporting neurons, regulating neurotransmitter concentrations, selectively transporting substances, inhibiting the entry of plasma macromolecules into the brain, and shielding the brain from neurotoxic impacts. Morphologically, the presence of tight inter-endothelial junctions and an absence of pinocytotic vesicles and fenestrations characterize the BBB [8,9]. Physiologically, cerebrovascular ECs form an exceptionally tight cellular barrier, displaying low ion permeability due to their high membrane resistance [10].

Tight junctions (TJ) interconnect CNS ECs, substantially impeding the extracellular passage of solutes. Moreover, ECs in CNS have a markedly reduced transcellular rate relative to peripheral ECs, which curtails vesicle-mediated solute transport [11]. This strict control over both paracellular and transcellular pathways results in a polarized cell structure, with

clear luminal and abluminal domains. This facilitates precise regulation over cellular transport properties between the blood and the brain [12].

ECs in CNS house a higher number of mitochondria as compared to ECs in other tissues. These mitochondria are postulated to be instrumental in generating ATP necessary for maintaining vital ion gradients that underpin transport functions. Additionally, CNS ECs express minimal levels of leukocyte adhesion molecules (LAMs), which limits immune cell infiltration into the CNS [13-15]. This combination of physical (like TJs and reduced transcellular transport) and molecular properties (such as efflux transporters, specialized metabolism, and reduced LAMs) coupled with specific nutrient transporters equips ECs to regulate CNS homeostasis.

Notably, understanding the varied genetic expressions of brain ECs in relation to ECs of other organs within the BBB context bears significant implications for future research endeavors. Previous investigations [16,17] have particularly cataloged the distinctive EC compositions within both the brain and kidneys. Two predominant subsets of ECs in the brain, namely EC (Ptn) and EC (Col6a1), identified by their preferentially expressed genes, were juxtaposed with renal endothelial cells. These studies unveiled the positive role of three pathways in these subsets in the context of stroke and depression [16,18]. Furthermore, two genes – Ube2g1 and Pdcd4 – emerged as pivotal players in stroke development [16]. Further research [19] explored the disparities in the immune complement system between these endothelial cells. Comparisons were made between the activation and regulation of the alternative complement pathway (AP) in human brain microvascular endothelial cells (BMVEC) and glomerular microvascular endothelial cells (GMVEC) under both tumor necrosis factor (TNF)-stimulated and unstimulated conditions. Results illustrated that BMVECs were markedly more resilient to TNF stimulation compared to GMVECs, showing superior control over AP activation. Additionally, activated protein C levels in BMVECs were

significantly higher than in GMVECs, hinting at a reduced propensity for thrombosis in BMVECs. This latter point is underlined by the strong correlation between decreased activated protein C levels and thrombosis. In terms of AP regulatory protein production, GMVECs were lacking compared to BMVECs [20]. Moreover, von Willebrand factor (vWF) expression in brain endothelial cells was relatively higher when compared with liver and kidney endothelial cells yet was on par with cardiac and lung endothelial cells. This could potentially elucidate the propensity of brain, heart, and lung endothelial cells to maintain hemostasis more efficiently [21].

Taken together, the ECs within the NVU possess singular attributes that set the BBB apart from other tissues. Their role is paramount, especially when studying brain ischemia-reperfusion injuries.

Mechanisms of Endothelial Cell Injury after Ischemia and Reperfusion in Stroke

In this section, the intricate mechanisms of endothelial cell damage resulting from ischemia-reperfusion, a critical event in stroke, were addressed. This damage stems from several key factors: disturbances in energy metabolism disrupting vital cellular processes, oxidative harm inflicted by reactive oxygen species (ROS), and a cascade of inflammatory responses and other pathways. These factors collectively contribute to the complex pathology of endothelial injury in the context of stroke, underlining the multifaceted nature of this medical condition (Figure 2).

1. Energy Metabolism Disturbances

During ischemic and hypoxic conditions, ATP synthesis in the mitochondrial respiratory chain is compromised [22]. With ATP depletion, the sodium-potassium pump on endothelial cells malfunctions, leading to the accumulation of sodium ions within cells and consequent cellular edema. Ischemia-induced acidosis further diminishes the intracellular pH, blocking sodium-hydrogen exchange pathways and activating sodium-calcium exchange. This results in an excessive influx of calcium ions into the cell, leading to intracellular calcium overload [23]. This overload activates phospholipase C and A2, degrading the cellular lipid membrane and producing free radicals. Concurrently generated fatty acids, prostaglandins, thrombin, leukotrienes, lysophospholipids, and platelet activators can cause localized inflammation, inflicting irreversible cellular damage [24]. Melatonin has been experimentally shown to alleviate endothelial cell damage by engaging the AMPK/SERCA2a pathway [25]. Furthermore, early-stage interventions with lithium chloride can reduce calcium overload in endothelial cells, primarily by inhibiting the release of inositol 3-phosphate-sensitive Ca2+ in the endoplasmic reticulum [26].

2. Destructive Effects of Reactive Oxygen Species (ROS)

Normally, the human body maintains a balance between oxidants and antioxidants. Physiologically, free radicals participate in cellular signaling, regulate cell growth, and help neutralize external threats, providing immune protection [27]. Pathological conditions, however, disrupt this balance, leading to excessive ROS production. These oxygen-derived molecules, either enzyme-induced or spontaneously formed, include both free radicals like superoxide and non-free radicals like hydrogen peroxide [28]. ROS can impose direct damage on cellular structures and indirectly initiate cell death through pathways such as the mitochondrial apoptosis and by influencing transcription factors, notably NF-κB [29]. Additionally, ROS can react with nitric oxide (NO) to form peroxynitrite, triggering apoptosis in endothelial cells [30].

3. Inflammatory Response

ECs play a pivotal role in neuroinflammation. Under hypoxic conditions, neurons and glial cells release detrimental signals, known as danger-associated molecular patterns (DAMPs)[31,32]. ECs, sensing these early alarms, facilitate inflammatory cell recruitment, driving an inflammatory cascade. This cascade compromises the integrity and permeability of the BBB and glycocalyx [33]. Local inflammation not only injures ECs but also perpetuates further inflammatory responses.

4. Synthesis and Secretion of Nitric Oxide (NO)

Nitric oxide (NO) is a small, gaseous signaling molecule synthesized in the body. Its primary role in the vascular system is to regulate blood vessel tone, ensuring proper blood flow and nutrient delivery to tissues [34]. NO acts as a potent vasodilator that expands blood vessels. This ability is crucial for maintaining the health of blood vessels and ensuring adequate blood supply to body tissues [35]. During ischemia (a reduction in blood supply), NO production within endothelial cells helps in dilating blood vessels, thus enhancing blood flow to deprived areas [36]. This vasodilatory action of NO also has an anti-inflammatory

effect, reducing potential tissue damage. Although NO has protective effects, an accumulation can be detrimental. In particular, an excess of NO in the mitochondria, the cell's energy powerhouse, can increase ROS production. ROS, in turn, can damage cellular structures, make the cell membrane leaky, and intensify tissue harm [37].

5. Injury of Endothelial Cells and Promotion of Neutrophil Aggregation

The endothelium lines the inside of blood vessels and plays a pivotal role in vascular health. When ischemia-reperfusion injury occurs, these cells can become damaged, which disrupts their normal function. Neutrophils are a type of white blood cell essential for immune responses [38]. Following endothelial injury, there is a surge of neutrophils in the affected area guided by a chemokine gradient. Activated by the damaged endothelial cells, neutrophils release certain chemicals known as vasoactive agents. These mediators can cause vasoconstriction, or narrowing of blood vessels, which can worsen local blood flow disturbances. This aggravates the initial ischemic injury, creating a vicious cycle of worsening tissue damage [39-42].

6. Apoptosis of Vascular Endothelial Cells

Cells can die in various ways. Ischemia-reperfusion injury can induce both necrosis (an uncontrolled form of cell death) and apoptosis (a regulated, programmed cell death)[43]. Apoptosis plays a dual role; while it helps in removing damaged cells, excessive apoptosis can be harmful. When vascular endothelial cells undergo apoptosis following ischemia-reperfusion injury, it contributes notably to the overall tissue injury. The programmed death of vascular endothelial cells can lead to intravascular thrombosis (blood clot formation within vessels) and tissue inflammation. These events can worsen the condition of the affected region, escalating damage and potentially leading to irreversible harm to the tissue [44].

Endothelial Cell Damage and Dysfunction underlying Ineffective Reperfusion

The consequence of stroke is not just about the cessation and resumption of blood flow to affected areas but is a multifaceted process that involves a cascade of physiological disruptions at the cellular and molecular levels. One of the consequences of stroke is the profound impact on endothelial cells, which play a pivotal role in maintaining cerebral health [45]. Firstly, the degeneration and death of cerebral endothelial cells post-stroke are well-documented [46]. As ischemia advances, these cells progressively deteriorate, manifesting first as simple edema and escalating to complete structural collapse, potentially resulting in hemorrhagic transformation. Such a progression is alarming as it does not just impair the local affected tissue but the entire blood recirculation. Moreover, the increased transcytosis compromises the BBB, making the brain more susceptible to external insults [47]. Further diving into the BBB, the disruption of TJs stands out [48]. Post-stroke, there is a disconcerting alteration in the structure and expression of these proteins, and this dysregulation has cascading effects. Proteolytic enzymes, for instance, accelerate the degradation of the BBB, further compromising its protective role. Beyond these specific cellular damages, the general microcirculation of blood in the brain suffers as well. Endothelial cells, while they act as the gatekeepers of vascular health, get overwhelmed by ischemic attack, leading to vasomotor impairment and reduced perfusion. While the multifaceted nature of these disruptions ensures that it is not just major vessels that suffer, micro-vessels, critical for the delivery of nutrients and oxygen also get caught in this disruptive storm [49]. Lastly, and perhaps most crucially, the event is the heightened inflammatory response post-stroke [50]. Endothelial cells, in tandem with glial cells, engage in a bidirectional inflammatory exchange, amplifying the outcomes of the stroke. The release of inflammatory mediators from the endothelial surface not only compromises its integrity

but also invites a surge of white blood cells into the brain. This invasion further exacerbates the post-stroke inflammatory response, amplifying ineffective reperfusion [51, 52].

1. Degeneration and Death of Cerebral Endothelial Cells

Degeneration Progression. Utilizing electron microscopy [53], a progression in ischemic brain endothelial cells over four distinct stages was identified. The initial stage exhibits endothelial edema accompanied by a reduced cytoplasmic content. Despite these changes, the endothelial cells maintain their barrier function, preventing the extravasation of tracers. During the second stage, endothelial cells begin to lose their protective barrier functions, allowing tracers to enter the cells. By the third stage, a complete loss of endothelial integrity is observed, allowing tracers to leak into the surrounding parenchymal tissue. Finally, in the fourth stage, the structural breakdown extends to the basement membrane, which can lead to hemorrhagic transformation of the ischemic tissue as red blood cells exude from the vessels. The significance of these findings is amplified in both ischemia-reperfusion and permanent ischemia animal models.

Endothelial Transport Dysfunction. Following cerebral ischemia, a significant increase in endothelial cell pits and vesicles, a crucial indicator of compromised integrity was observed [50]. This escalation in transcytosis is linked to a surge in the permeability of the BBB [50]. Recent animal model studies illuminated that the increase in BBB permeability post-stroke was associated with both transcellular and paracellular transport mechanisms [54]. In specific experiments, mice genetically modified to lack Caveolin1 exhibited a decline in transcellular permeability in their cortical vessels post-t-MCAO, highlighting the distinct regulatory pathways governing each transport mode. Furthermore, the influence of stroke extends to ion channels on endothelial cells, inducing dysfunction in mechanisms like the Na+/K+-ATPase, especially when confronted with elevated external potassium activity [55].

2. Breakdown of Endothelial Cell Tight Junctions Leading to BBB Dysfunction

Molecular Composition of TJs. TJs are complex structures crucial for maintaining cell-cell adhesion and permeability control in endothelial tissues (Figure 3). They are primarily composed of proteins from the claudin (CLDN) family, the MARVEL protein (TAMP) family, the Junctional adhesion molecules (JAM) family, and the Zonula occludens (ZO) family [56]. Among these, CLDN-5 has been spotlighted as a fundamental component, especially given its predominant mRNA expression when compared to other CLDN molecules [57, 58]. The important role of the TAMP family in augmenting the intricacy of TJs has been emphasized in recent studies, underscoring its crucial role in bolstering resilience of the BBB [59, 60]. Additionally, JAM-A interactions, both cis and trans, are not standalone processes, while they require intricate interactions with TJ scaffold proteins and other auxiliary molecules for their functionality [61-63]. ZO-1 plays an irreplaceable role in this orchestra, providing stabilization to CLDN-5 expression and ensuring the successful assembly of TJs in microvascular endothelial cells [64].

Impact of Cerebral Ischemia on TJs. The impacts of cerebral ischemia resonate deeply within the molecular structure of TJs. Ischemic conditions and accompanying inflammation lead to profound structural alterations in TJ proteins, manifesting as phosphorylation events and other post-translational modifications [65]. This structural turmoil is not the only casualty and ischemia also triggers regulatory disruptions in protein distribution and expression within TJs. For instance, post-stroke abnormalities in the TJ chain become clearly apparent around 48-58 hours after a transit MCAO, coinciding with a second peak of escalated biphasic BBB

permeability [66]. These phenomena are not isolated; they hint at a consistent pattern of delayed TJ remodeling that endothelial cells undergo in response to various ischemic events. Beyond these disruptions, proteolytic enzymes like tissue plasminogen activator (tPA), matrix metalloproteinase (MMP), cathepsin, and heparinase were also inducted. These enzymes spearhead the degradation of the BBB's extracellular matrix (ECM), further dismantling TJs via integrin-driven mechanisms [67].

3. Microcirculation Disorders with Endothelial Cell Injury

Dynamics of Microcirculation Dysfunction. The intricacies of microcirculation dynamics are puzzled into disarray following an ischemic event. The endothelial cells, stewards of vascular health, bear the brunt of these disruptions. The manifestations of this dysfunction are multifold: 1) Arterioles betray signs of impaired vasomotor functionality, characterized by endothelium-dependent and independent dilations, with an increased susceptibility to thrombosis events; 2) Capillaries show patterns of contraction, obstruction, and diminished perfusion. This results in compromised delivery of essential oxygen and nutrients to the already beleaguered ischemic tissue; 3) In venules, a marked endothelial barrier dysfunction becomes apparent. This soon progresses to severe vascular injuries, facilitating the extravasation of blood cells. It's also noteworthy that post-ischemia/reperfusion (I/R) conditions trigger an overexpression of adhesion molecules in venules. This increases white blood cell adhesion and migration, eventually culminating in further tissue damage [68].

Factors Influencing Microcirculation. The turbulence in post-stroke microcirculation is not random but coordinated by a slew of specific factors. For instance, the exerted pressure from cerebral edema can directly impact capillary functionality [69]. Another player in this narrative is the pericyte, a cell type that plays a regulatory role in adjusting the diameter of

microvessels, thereby influencing blood flow [70]. There are conflicting thought as to whether ischemic damage is due to pericyte contractions in the early stages of ischemia or due to the increase in ROS by the Nox4 enzyme pathway which leads to the destruction of pericytes[71]. As studies continue to explore the exact involvement with pericytes, it ultimately leads to the compromise of the BBB integrity. As these factors converge, the outcome is a suboptimal recanalization process. While large vessels might find a way to limp back to functionality, the capillaries could be left stranded, prone to further pathological alterations.

4. Inflammatory Processes Associated with Endothelial Cells

Significance of Endothelial-Glial Interactions. The consequence of stroke is characterized by an intense inflammatory response, triggering both endothelial and glial cells into action. As these cells become activated, they are entwined in a dynamic of close interactions. These interactions are not just passive observations, but they indicate the potential of these cell types to induce specific functionalities in each other [72]. For example, damaged neural tissues stimulate microglia and astrocytes into releasing a barrage of cytokines and chemokines. This chemical storm, in turn, actively stimulates endothelial cells, establishing a feedback loop [73]. This intricate ballet emphasizes a reciprocal inflammatory communication between endothelial cells and their glial counterparts, with potential ramifications for the post-stroke inflammatory landscape [74].

Repercussions of Inflammation on the Endothelial Surface. At the front of the inflammatory response, the endothelial glycocalyx layer stands sentinel. Inflammatory conditions force this layer into releasing a litany of inflammatory mediators. The downstream effects are profound, including structural weakening of the glycocalyx, culminating in the

exposure of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), leading to inflammatory cells to adhere [75-78]. Beyond this, endothelial cells express selectins that streamline the process of white blood cell rolling. This intricate dance of chemokine interactions promotes the trans-endothelial migration of these white blood cells into the tissue [71]. As these cells gain entry, they release proteases, reactive oxygen species (ROS), and other inflammatory compounds, exacerbating reperfusion injury and complicating the post-stroke recovery process.

Taken together, endothelial cell degeneration, encompassing stages from mere cellular edema to total structural collapse, hinders the restoration of optimal blood flow during reperfusion. Compromised TJs in the BBB, driven by post-stroke alterations in protein structures and expressions, leave the brain vulnerable even when reperfusion is initiated. Furthermore, the disruption of microcirculation, marked by vasomotor impairments and reduced capillary perfusion, challenges the very essence of effective reperfusion by obstructing efficient nutrient and oxygen delivery. Coupled with this, the heightened post-stroke inflammatory response, characterized by intense endothelial-glial interactions and the invasive cascade of white blood cells, exacerbates ineffective reperfusion. Although therapies like thrombolysis or thrombectomy can open blocked vessels, the intricate cellular and molecular disruptions detailed above can still culminate in ineffective reperfusion. Addressing these underlying endothelial cell damages and dysfunctions becomes pivotal in truly aiding post-stroke recovery.

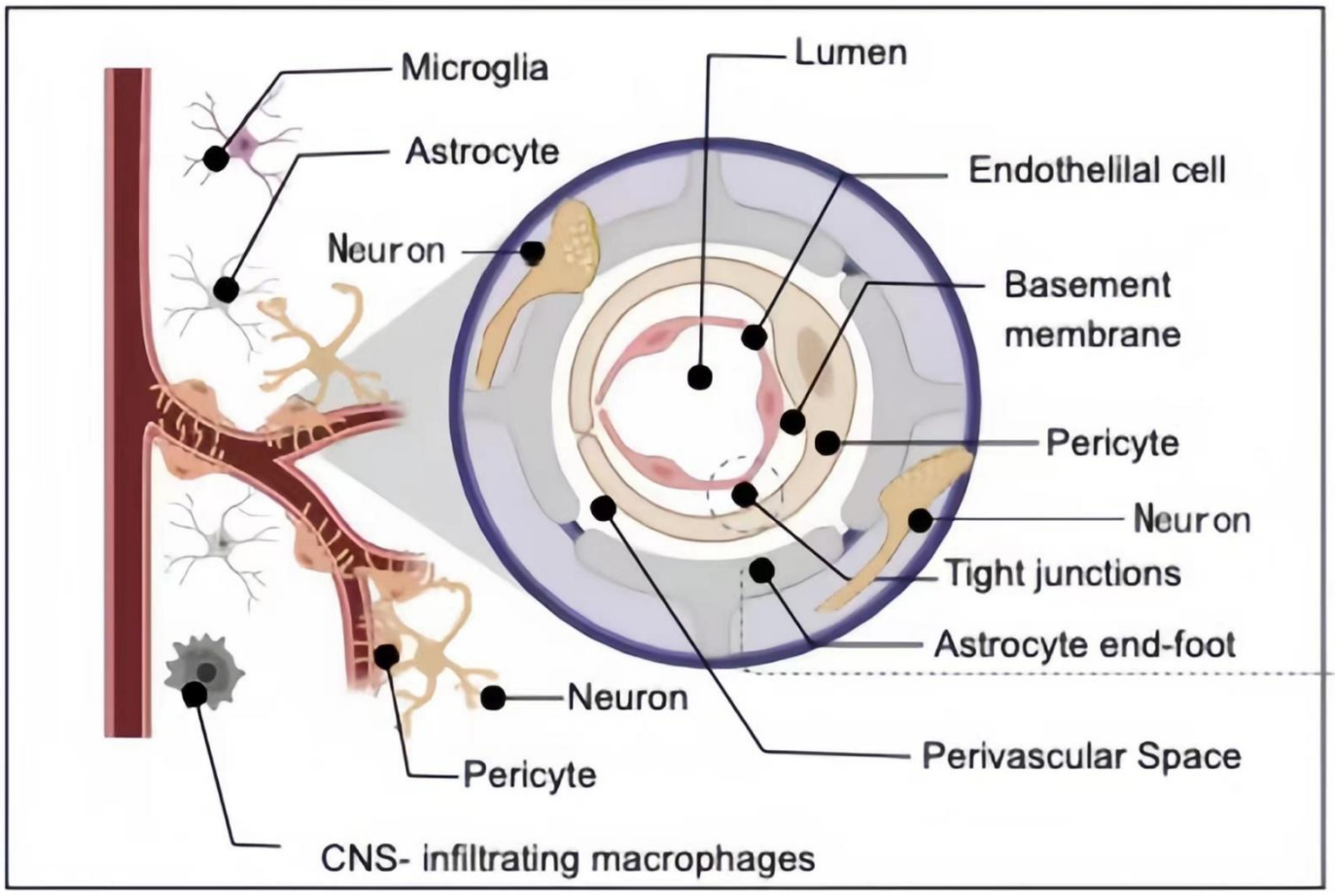
Conclusion and Future Direction

Ineffective reperfusion represents a significant challenge in the realm of

neurointerventional medicine, with injury to the BBB being a central concern. This review highlights the significance of brain endothelial cells, especially during episodes of stroke with inefficient reperfusion. This article addresses the distinguishing features of brain endothelial cells in comparison to their counterparts in other tissues, elucidating their pathologies, dysfunctions, and inflammatory reactions post-stroke.

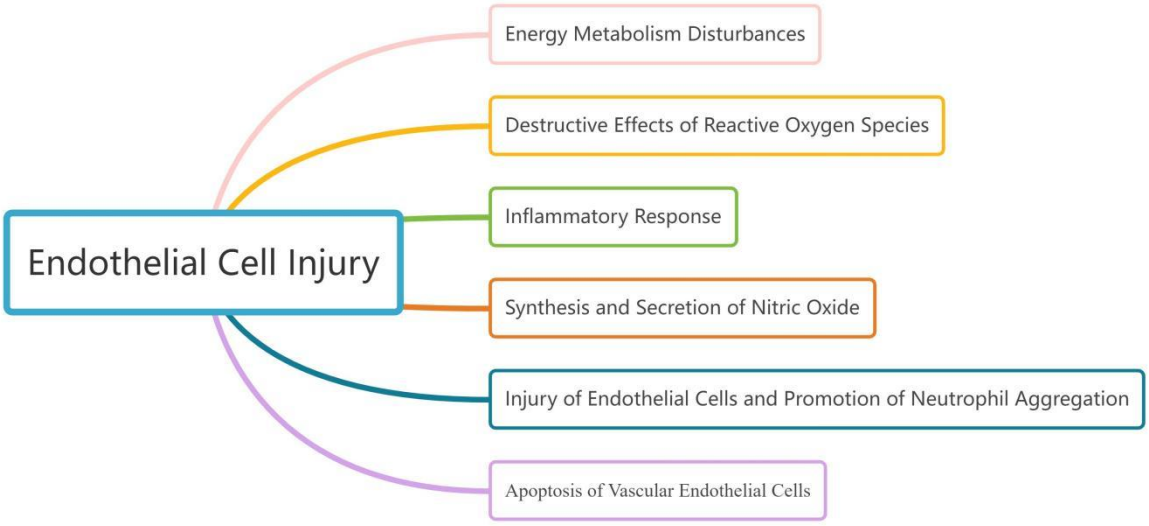
Evidently, brain endothelial cells occupy a pivotal position in neuroprotective therapeutic strategies. The ongoing research centered on these cells holds tangible promise. As we gaze towards the future, the authors speculate that drawing analogies between brain endothelial cells and endothelial cells from organs with pathological environments akin to cerebral ischemia-reperfusion injuries holds substantial merit. All these connected factors, though distinct in nature, collectively contribute to a view where, even with interventions like thrombolysis or thrombectomy, the desired reperfusion outcomes remain elusive.

Figure 1. Composition of neurovascular units



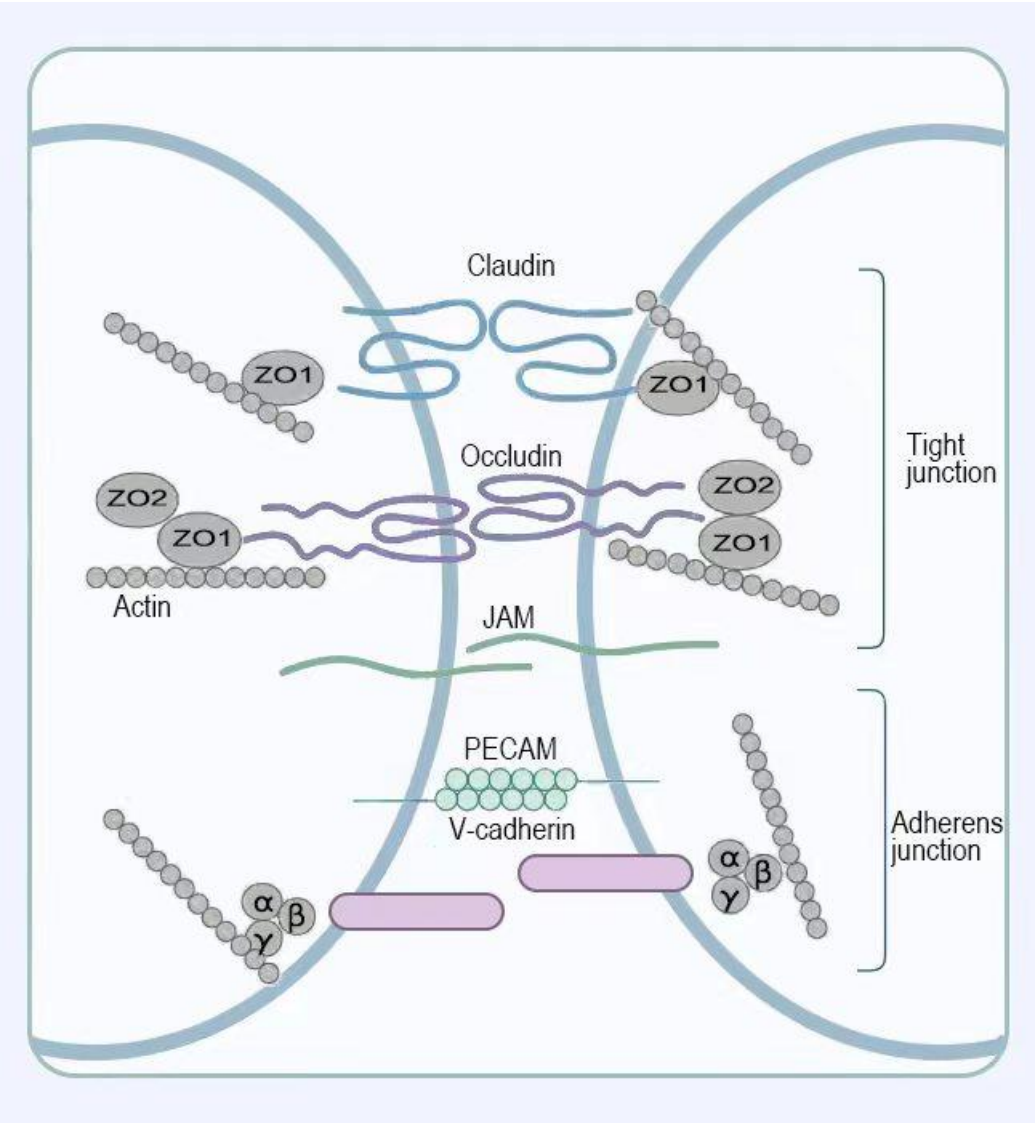
Diagrammatic representation of the neurovascular unit (NVU) in a cross-section, showcasing the intricate cellular and structural components which ensure the functional integrity of the blood-brain barrier. The elements highlighted include neurons, astrocyte, microglia, pericyte, endothelial cell, tight junctions, and the basement membrane. Neurons distinctly connect with blood vessels and other cells of the NVU. Endothelial cells forming the blood vessels are encased by a basement membrane and are bound by tight junction proteins. Astrocytes contact with both pericytes and endothelial cells, while pericytes are situated between the end feet of astrocytes and endothelial cells. Microglia are scattered evenly throughout the brain, and occasionally extend a process directly to the vasculature.

Figure 2. Mechanisms of Endothelial Cell Injury



Conceptual diagram illustrating various factors leading to endothelial cell injury. The highlights on the roles of energy metabolism disturbances, destructive effects of reactive oxygen species, inflammatory response and other pathways are shown. The diagram also emphasizes the synthesis and secretion of nitric oxide, injury mechanisms to endothelial cells, promotion of neutrophil aggregation, and apoptosis of vascular endothelial cells.

Figure 3. The Tight and Adherens Junctions



Schematic representation of cell-cell junctional complexes, illustrating key components of tight junctions and adherens junctions. Claudin, occludin, and junction adhesion molecules (JAMs) are the transmembrane proteins, and ZO-1, ZO-2, and cadherin are the cytoplasmic proteins. This figure emphasizes their role in maintaining cell structure. Among them, CLDN-5 has the most significant function. JAM-A require interactions with TJ scaffold proteins and other auxiliary molecules for their functionality. ZO-1 provides stabilization to CLDN-5 expression and ensuring the successful assembly of TJs in microvascular endothelial cells. Adherens junctions have a similar organization to TJs. They contain

cadherins, alpha-actinins, mostly responsible for the adhesion, involved in supporting cadherin association and regulating out-in signaling processes, such as cadherin contact with Platelet endothelial cell adhesion molecule-1 (PECAM-1).

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