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REVIEW ARTICLE

The Role of VE-cadherin in Blood-brain Barrier Integrity under Central Nervous System Pathological Conditions

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Abstract: The blood-brain barrier (BBB) is a layer between the blood circulation and neural tissue. It plays a pivotal role in maintaining the vulnerable extracellular microenvironment in the neuronal parenchyma. Neuroinflammatory events can result in BBB dysregulation by disturbing adherens junctions (AJs) and tight junctions (TJs). VE-cadherin, as one of the most important components of the vascular system, is specifically responsible for the assembly of AJs and BBB architecture. Here, we present a review, which highlights recently available insights into the relationship between the neuroinflammation and BBB dysregulation. We then explore the specific interaction between VE-cadherin and BBB. Finally, we discuss the changes of VE-cadherin with different neurological diseases from both experimental and clinical studies. An understanding of VE-cadherin in BBB regulation may indicate that VE-cadherin can partially be a biomarker of neuroinflammation disease and lead to novel approaches for abating BBB dysregulation under pathological conditions and the opening of the BBB following central nervous system (CNS) drug delivery.

Keywords: Blood-brain barrier, endothelial cells, VE-cadherin, neuroinflammation, central nervous system, adherens junctions.

1. INTRODUCTION

The blood-brain barrier (BBB), which plays a pivotal role in creating a unique and stable microenvironment for neuron activity, is one of the most important components for the central nervous system (CNS) [1]. The BBB is composed of a monolayer of endothelial cells (ECs), which line the capillaries that restrict the entry of proteins and inflammatory cells into the brain for the protection of the brain microenvironment [2, 3]. A wide range of CNS pathologies, such as cerebral ischemia, trauma, or multiple sclerosis can disrupt BBB integrity, which can induce edema, inflammation, and cell death. The occurrence of BBB integrity disruption, accompanied by the transmigration of numerous inflammation molecules, leads to secondary injury to neurons [4].

On the cellular level, cell-cell junctional complexes, such as tight junctions (TJs) and adherens junctions (AJs), located in the apical region of ECs membranes, almost obliterate paracellular space (Fig. **1**) [5-7]. CNS pathologies-induced down-regulation of the junctional complex will result in the entrance of molecules into the brain parenchyma through the paracellular pathway and represent vital components in BBB integrity. VE-cadherin, an endothelial-specific transmembrane protein, is well known for its function in promoting early stages of vascular connection and fusion [8, 9]. In recent years, growing evidence points to a role of VE-cadherin in the maintenance of cell-cell junction stabilization and regulation of vascular barrier integrity [10-13].

Considering the essential role that VE-cadherin plays in vascular permeability, this review will focus on VE-cadherin organization in BBB to highlight the functional activity of this molecule. The role of VE-cadherin in regulating BBB permeability will be elucidated, as well as the intracellular signaling involved in CNS pathologies that result in BBB leakage.

2. BBB STRUCTURE AND FUNCTION

The BBB, which is primarily composed of brain endothelial cells, constitutes a "diffusion barrier" which prevents the entry of a protein-rich exudate and thereby creates a vulnerable extracellular microenvironment in the neuronal parenchyma [14]. Unlike the low density of junctional complexes in ECs of other organs' endothelial cells, cerebral endothelial cells have a higher density of junctional complexes between adjacent ECs, which restrict diffusion of most molecules. In addition, cerebral endothelial cells have lower rates of transcytosis and active transporters [15, 16]. Unlike other organs' capillary beds, brain parenchyma has the largest peri-

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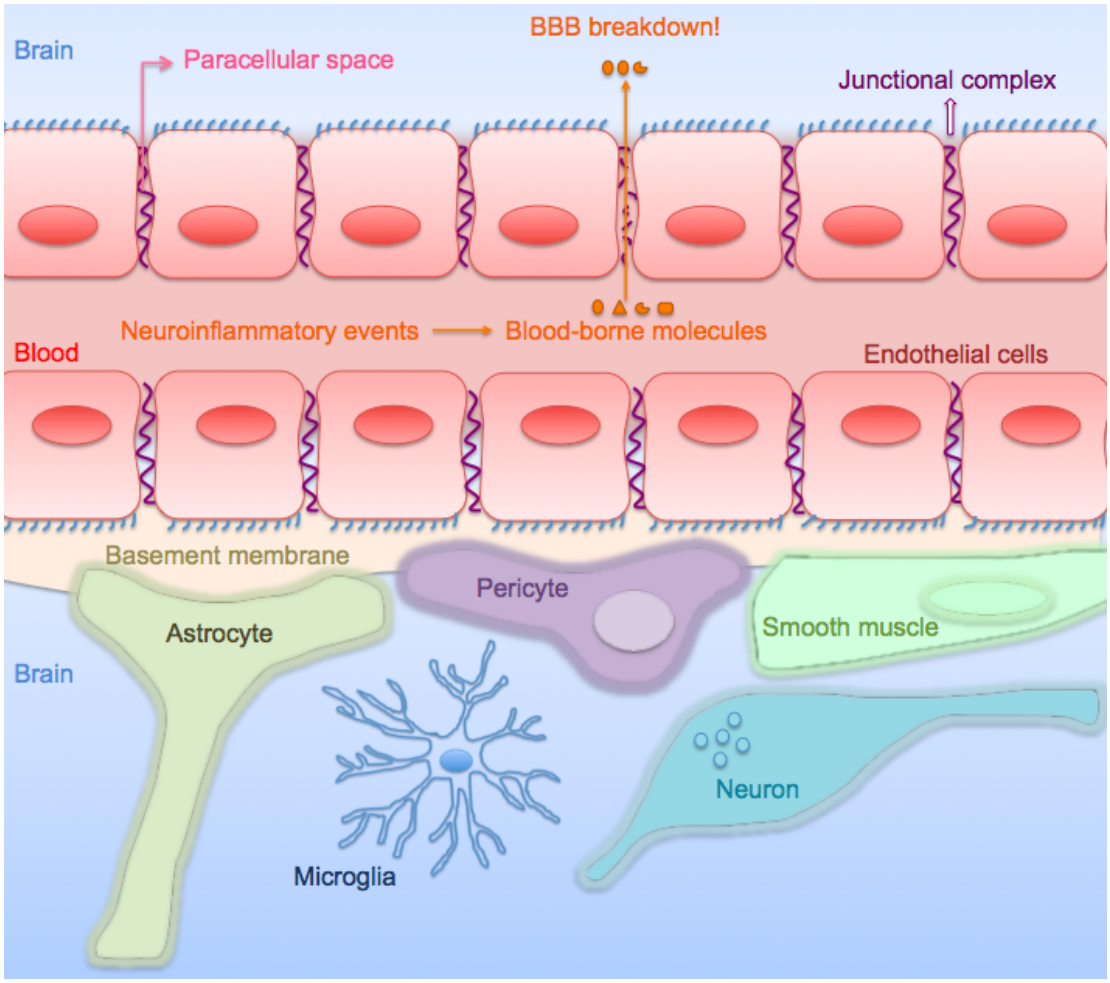

Fig. (1). Cellular constituents of the blood-brain barrier. The blood brain barrier exists between the lumen of the cerebral blood vascular system and brain parenchyma. Cerebral capillary endothelial cells are surrounded by basement membrane. Pericytes and the endfeet of astrocytic glial cells are enclosed within the endothelial basement membrane, which provides cellular communication to the endothelial cells. Microglial cells and neurons interact with core elements of the BBB, which influences the barrier layer functions. Junctional complex exists in the paracellular space. When neuroinflammatory events occur, which releases blood-borne molecules to the brain through paracellular space, this causes the BBB to breakdown.

cytes coverage which surrounds the abluminal surfaces of the cerebral vascular system [17]. Recent work suggests pericytes, together with cerebral endothelial cells, present a specific synergistic function in the regulation of vascular integrity through releasing chemokines, pro-inflammatory cytokines, reactive oxygen species, and matrix metalloproteinases (MMPs) [18]. The role of the endfeet of astrocytic glial cells enwrapping the abluminal side of the cerebral vascular system is outlined, which affects the BBB phenotype of the cerebral endothelial cells [19]. The endfeet of astrocytic glial cells has many functions, such as distributing energy substrates to neurons, maintaining cerebral potassium balance, controlling immune responses, and regulating BBB integrity by promoting the differentiation of ECs and formation of junctional complexes [20]. The basement membrane is made up mostly of macromolecular components, such as collagen, integrins, fibronectin, heparan sulfate, entactins, laminin, and dystroglycans. Basement membrane existing in the perivascular space can build up a cerebral microarchitecture, which further regulates the intercellular crosstalk between members of a neurovascular unit [14, 21]. Taken together, ECs are essential for normal function of the BBB, while other constituent cell types also contribute to the BBB's integrity (Fig. **1**).

The functions of BBB are numerous, including: (1) distributing essential nutrients to the brain by specific transport systems and mediating the release of many waste molecules; (2) regulating the movement of ions and fluid between the blood and cerebral parenchyma, which may otherwise disrupt the vulnerable balance of the cerebral microenvironment; and (3) keeping the pools of neurotransmitters and neuroactive agents that act in the CNS and peripheral tissues and blood separate, so that the same agent can be used in the two systems without 'crosstalk' [22-24].

Although recent studies revealed the structural formation of the BBB, the underlying molecular mechanisms of many of the processes, as well as how different molecules cooperate to assemble this vascular network, remains unclear.

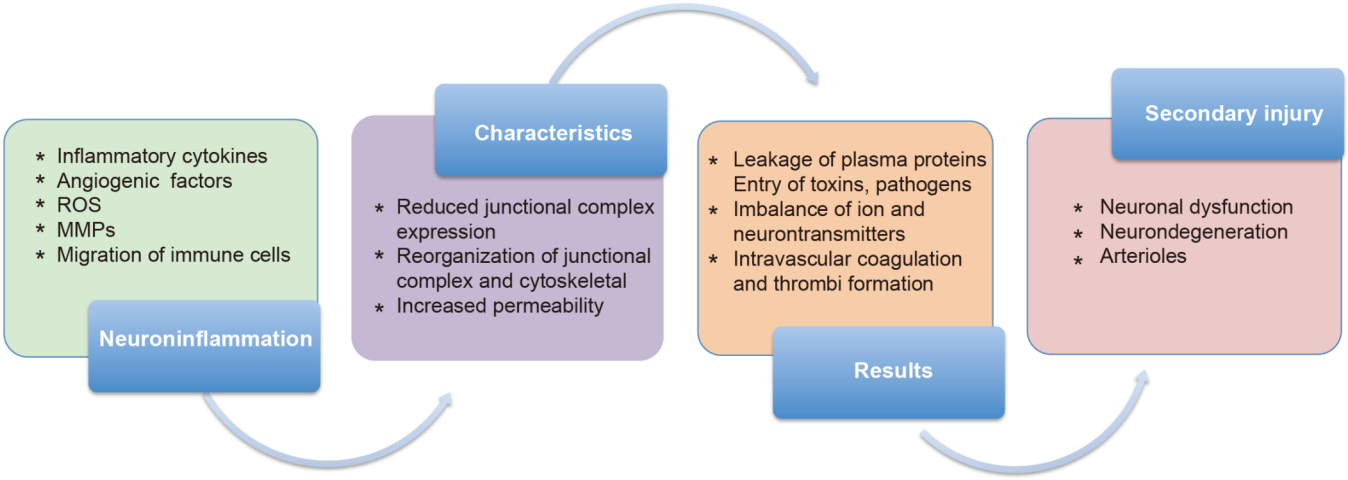

Fig. (2). The process of neuroinflammatory events. Neuroinflammatory events can induce many factors to increase, such as inflammatory cytokines, angiogenic factors, ROS, and MMPs. These factors can disrupt the BBB integrity by reducing and reorganizing the junctional complex, and increasing the cerebral endothelial permeability. BBB breakdown aggravates impairment of BBB functions, ranging from leakage of plasma proteins to intravascular coagulation. BBB breakdown can lead to secondary injury which, in turn, aggravates neuronal dysfunction and neurodegeneration.

3. BBB DYSREGULATION IN CNS PATHOLOGICAL CONDITIONS

Brain microvascular endothelial cells act as an "immune system barrier" to limit the entrance of pro-inflammatory proteins and cells [4]. However, various pathological conditions, such as cerebral ischemia, trauma, or multiple sclerosis will lead to BBB dysregulation, which results in the transmigration of numerous activated pro-inflammatory cells and entry of protein-rich influx [15]. Chronic dysfunction of BBB can enhance overall cerebral pathology and induce persistent neurological deficits through activating the coagulation pathway, which leads to thrombi formation in cerebral venules and, to a lesser extent, arterioles [25]. Platelet aggregation and pro-inflammatory cells extravasation observed in cerebral venules exert their function in significantly decreasing cerebral blood flow and further contribute to secondary ischemic injury (Fig. **2**) [26].

3.1. Inflammatory Response

Inflammation seems to play a critical role in neurodegenerative disorders, and occurs after disease onset and propagates progression. Microglia, neurons, astrocytes, and endothelial cells can release pro-inflammatory cytokines and chemokines, such as interleukin (IL)-1β, tumor necrosis factα (TNF-α), IL-6, or vascular endothelial growth factor (VEGF) [27]. These major proinflammatory molecules have been suggested to promote BBB permeability [28].

TNF binds to the extracellular domains of TNF receptor 1 (TNFR1) and collects TNFR1−associated death domain protein (TRADD) to the cytoplasm death domain of the TNFR1. TRADD, in turn, recruits the serine/theonine kinase receptor-interacting protein (RIP) 1 and TNFR-associated factor 2 (TRAF2). The complex initiates various kinase pathways that activate activator protein 1 (AP1) and transcription factors nuclear factor-κB (NF-κB) [29]. IL-1 can bind with its own receptor (IL-1R) which, in turn, activates myeloid-differentiation factor 88 (MYD88)-mediated, the same transcription factors as TNF [30]. NF-κB and AP1 also mediate new gene transcription and new protein translation. Disruption of BBB in response to TNF and IL-1 are triggered in an NF-κB and protein-translation-dependent manner [31]. They increase the permeability of the BBB by stimulating vascular endothelial cells to reorganize their cytoskeletons, and thereby form gaps between adjacent cells [32].

In the inflammatory infiltration of patients, who are infected with bacterial meningitis, accumulated VEGF in brain parenchyma has been detected, which suggests that pro-inflammatory cells release VEGF during disease progression, thus leading to BBB permeability [33]. VEGF can interact with two plasma membrane receptors, kinase insert domain-containing receptor (KDR) and fms-like tyrosine kinase (FLT-1), which induce their autotyrosine phosphorylation and downstream mediators. VEGF has been demonstrated to disrupt the integrity of cultured brain microvascular endothelium by altering junctional proteins occludin, ZO-1, and actin filament distribution in the cellular monolayers [34, 35].

3.2. Oxidative Stress

Oxidative stress plays a critical role in BBB breakdown in neurological diseases [36, 37]. Oxidative stress activates local migration of microglia, and upregulation of inflammatory mediators and reactive oxygen species (ROS) that can induce oxidation of proteins. It can also activate MMPs, DNA and lipids, as well as reorganize AJ and cytoskeleton [38, 39]. Depletion of the endogenous antioxidant glutathione induced by oxidative stress will induce paracellular permeability of BBB through leakage of small molecular-weight markers and redistribution of occludin and ZO-1 [40]. Increased production of ROS can induce peroxidation of membrane polyunsaturated fatty acids, which results in BBB permeability [41]. In human studies, it was found that the concentrations of MMP-9 in plasma were significantly

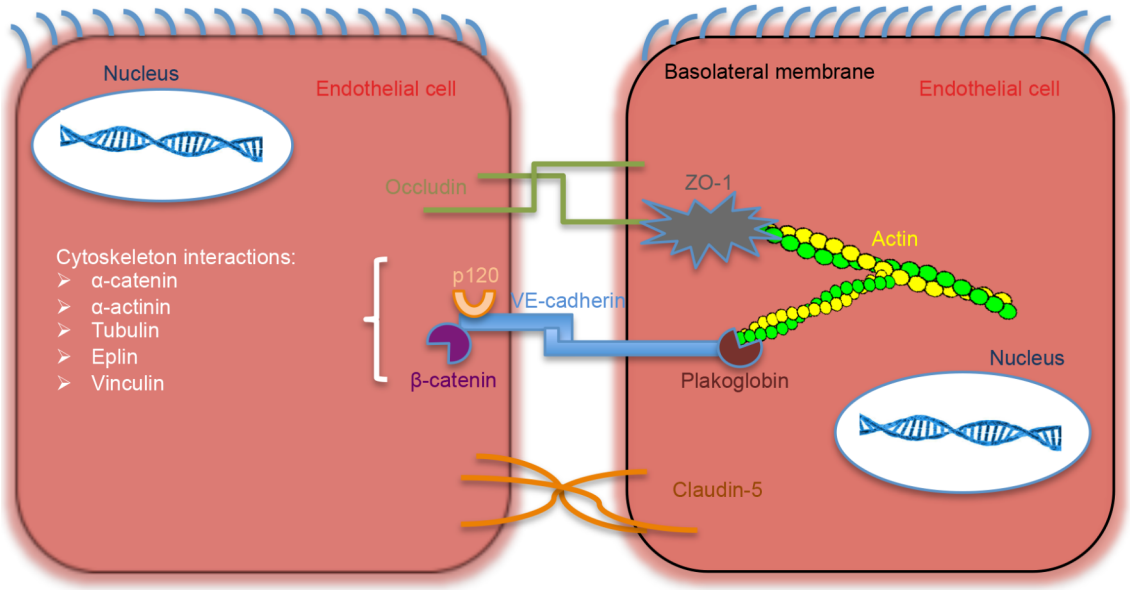

Fig. (3). Molecular composition of cerebral endothelial cells junction complex. Claudin-5 and occludin are the main transmembrane molecules of tight junctions mediating endothelial cell integrity. Occludin binds to the cytoskeleton *via* ZO-1. Contact in adherens junctions is established mainly through VE-cadherin. VE-cadherin interacts with the cytoskeleton *via* cytoplasmic anchor proteins, β-catenin, plakoglobin (γ-catenin), and p120 that belong to the Armadillo family. β-catenin and plakoglobin bind to α-catenin, α-actinin, tubulin, eplin and vinculin, which links the cadherin/catenin complex to the F-actin-based cytoskeleton.

correlated with the outcome of cerebral ischemia patients [42]. Using brain microvascular endothelium, MMP-9 treatment significantly down-regulated the expression of tight junction ZO-1, indicating an increase in permeability [43]. Interestingly, *in vivo* studies have demonstrated that MMP-9 gene knockout mice markedly decreased transient focal cerebral ischemia-induced BBB permeability and edema compared to wild-type mice [44].

The molecular basis of neuroinflammation is not completely understood, and pharmacological intervention to prevent or rescue BBB dysfunction in neuroinflammation diseases constitutes a challenging task. However, new prospects for potential therapies may be discerned from studies of endogenous mediators regulating BBB integrity.

4. VE-CADHERIN

Recent efforts to elucidate the molecular structure of junctional complex on BBB function have mainly focused on TJs. However, cadherin/catenin complex, as adherens junction proteins, regulate adhesion of the ECs, which contributes to the overall junction arrangement [45]. Cadherins, a class of transmembrane proteins, are receptors dependent on calcium, which form adherens junctions to bind with neighboring cells [46-48]. Cadherins can act with the actin cytoskeleton *via* cytoplasmic intermediary proteins, β-catenins, γ-catenins (also known as plakoglobin), and p120 that belong to the Armadillo protein family [49-52]. Cadherin expression at the plasma membrane is associated with catenin and p120 through their binding to the cadherin/catenin-binding domain and juxtamembrane domain, respectively. With this binding, cadherins create zipper-like structures that maintain stable adhesion between brain endothelial cells. In the AJs, N-cadherin and VE-cadherin are both expressed in endothelial cells. However, unlike N-cadherin expressed in several other cell types, such as neural cells and mesenchymal cells, VE-cadherin is exclusive to ECs and helps them to communicate with other cells of the same type [53, 54]. VE-cadherin can also bind to p120 *via* its short cytoplasmic tail, which determines the distribution of VE-cadherin at AJs and maintains endothelial integrity (Fig. **3**) [6, 53]. Thus, the location and structure of VE-cadherin make it susceptible to inflammatory factors generated in neuroinflammatory events.

4.1. VE-cadherin Function

During the 7.5 embryonic day mark, VE-cadherin transcripts have been detected in mesodermal cells of the yolk-sac mesenchyme [55, 56]. Due to this, VE-cadherin contributes to the maturation and remodeling of embryonic angiogenesis [8]. Using VE-cadherin knock-out mice, it was found that there were obvious collapse and regression of the vascular system, which led to early embryonic lethality [57]. In a zebrafish model, VE-cadherin was involved in vascular connections and the inhibition of sprouting activity. Even an incomplete deletion of VE-cadherin caused instability in the vascular system [11]. In a fish model with no functional VE-cadherin expression of the vessels, the early sprouting vessels failed to anastomose correctly [58]. In adult mice, binding VE-cadherin's extracellular domain with functional-blocking antibodies resulted in impaired angiogenesis and increased microvascular dysfunction [59, 60].

All of these findings indicate the importance of VE-cadherin as a critical component of the vascular system. However, VE-cadherin also plays an essential function in the BBB. The deletion of VE-cadherin in mice disrupted TJ integrity and altered localization of ZO-1, claudin-1, and claudin-4 by activating Rac and protein kinase C [61].

4.2. Signaling of VE-cadherin

VE-cadherin can activate signal molecules with a role in cytoskeleton organization. The signaling transduced by VE-cadherin is complex and varies under different conditions [62]. For vascular integrity, the Rho GTPase family has an important function in mediating the permeability of the endothelial barrier under both physiological and pathological conditions [63]. It has been shown that Rac1 (Ras-related C3 botulinum toxin substrate 1) and Cdc42 (cell division control protein 42 homolog) contribute to the formation of filopodia and lamellipodia, which improves vascular barrier integrity. However, the member A (RhoA) organizes the formation of stress fibers when the cell contracts and can impair vascular barrier integrity [64, 65]. By recruiting upstream effectors of Rho GTPase to the sites of cell-to-cell adhesion, VE-cadherin can make an essential space to maintain Rho GTPase activities [66].

In addition to VE-cadherin function in cell-to-cell adhesion, catenins have also been detected to mediate intracellular signaling. The complex of VE-cadherin-β-catenin is able to enrich α-catenin, which reduces Arp 2/3 activity (Fig. **3**) [67, 68]. The reduction of Arp2/3 activity is associated with actin branching and assembly, which affects cell-to-cell stability and contact [69]. VE-cadherin binding partners, β-catenin and p120-catenin, can be dissociated under stimulation and then regulate transcription by translocating to cell nucleus [70]. β-catenin, an important component of Wnt signaling, can transduce Wnt proteins to bind with frizzled family receptors and enter the nucleus to form a complex with transcription factors of TCF/LEF. This complex induces activation of Wnt target genes transcription process [71, 72]. As previously discussed, VE-cadherin can recruit catenins to the cell membrane to inhibit nuclear signaling. When p120-catenin translocates the nucleus, it binds with the transcriptional repressor Kaiso (a POZ/zinc-finger family member) and activates Kaiso-mediated gene repression [73, 74].

5. VE-CADHERIN AND BBB DYSREGULATION

VE-cadherin is a structural component for brain vascular integrity, and therefore is the primary target of BBB breakdown signaling events.

5.1. VE-cadherin Internalization

VE-cadherin is a highly dynamic adhesion molecule, and its availability at the plasma membrane is associated with cell-cell adhesion and junctional complex function. These two results could both contribute to endothelial cell permeability [75]. Internalization of VE-cadherin is believed to reduce the amount of the protein at junctions, which affects endothelial barrier function. Under neuroinflammatory conditions, junctions are disrupted and vascular integrity is compromised. This process, however, appears to be complex. Several signal pathways have been demonstrated to affect VE-cadherin internalization, such as PI3K signaling that is related to the loss of vascular barrier. Class I PI3Kα triggers TNFα signaling to cause VE-cadherin destabilization [76]. Additionally, new findings showed that IL-1β binding to its receptor can activate an MYD88-ARNO-ARF6 cascade to disrupt VE-cadherin localization which, in turn, increases vascular stability [13].

Recently, numerous studies have reported that the binding of p120-catenin to VE-cadherin could inhibit induced VE-cadherin endocytosis [77]. In addition, some amino acids within the core p120-VE-cadherin binding region have also been linked to VE-cadherin uptake [78]. A more thorough understanding of the precise molecular mechanisms involved in VE-cadherin internalization is urgently needed. However, it is believed that this process occurs through the masking of an endocytic signal sequence, thereby preventing VE-cadherin internalization and degradation, and thus stabilizing the junctional complex [78, 79].

5.2. VE-cadherin and Actin Cytoskeletal Remodeling

VE-cadherin includes two main domains: a transmembrane domain-N-terminal extracellular domain, and a short cytoplasmic tail-C-terminal domain. The cytoplasmic tail of VE-cadherin can bind with β-catenin and γ-catenin. γ-catenin and β-catenin can be associated with α-catenin, which further regulates the actin cytoskeleton [80]. As previously discussed, the VE-cadherin/catenin complex can activate signal molecules with roles in cytoskeleton organization. The complex interacts with actin filaments, which regulated initial junction formation, maintenance of junction integrity, junction maturation, and junction remodeling in neurological diseases [62]. Although interaction between actin cytoskeleton and cadherin-catenin complexes remains a topic of debate, a recent study suggests that focal adherens junctions (FAJs), junctions that are molecularly and phenotypically distinct subsets of VE-cadherin adhesions, are connected with radial F-actin bundles [81]. In the remodeling of endothelial cell-cell adhesions, inflammatory cytokines and oxidative stress-induced signals stimulate the separation of FAJs from stable AJs. FAJs couple to radial F-actin bundles, which are marked by the mechanosensory protein Vinculin, to disrupt endothelial cell integrity [81].

5.3. VE-cadherin Phosphorylation

The cytosolic tail of VE-cadherin harbors various tyrosine residues regulating the *in vitro* transmigration of leukocytes. This indicates that tyrosine phosphorylation is important in the regulation of endothelial junctions [75]. Under CNS pathological conditions, inflammatory cytokine and oxidative stress components, such as histamine and VEGF, were shown to enhance VE-cadherin phosphorylation. This resulted in dissociation of the VE-cadherin/catenin complex which, conversely, hampered vascular permeability [82]. The vascular endothelial protein tyrosine phosphatase (VE-PTP), an endothelial-specific transmembrane protein, can be associated with the VE-cadherin extracellular domains supporting VE-cadherin adhesion in endothelial cells [83]. After BBB breakdown, leukocytes binding to vascular endothelial cells in the brain leads to the separation of VE-PTP from VE-cadherin, which induces endothelial cell permeability [84]. An *in vivo* study suggests that inhibition of the loosening of the VE-PTP/VE-cadherin complex attenuated VEGF-induced vascular permeability [85]. Besides VE-PTP, several other molecules that are associated with VE-cadherin phos-

phorylation are essential for the regulation of brain vascular endothelial cell integrity [86-88]. Recent studies have reported that the five putative phosphor-tyrosine sites (Y645, Y658, Y685, Y731, and Y733) are involved in decreasing endothelial cell monolayer integrity [89, 90]. In addition, serine phosphorylation (S665) of VE-cadherin has been reported to regulate AJ assembly [91, 92]. However, the mechanisms underlying VE-cadherin phosphorylation-induced vascular permeability are not fully understood. Furthermore, the level of importance of site phosphorylation, and how to target it for therapy through dephosphorylation, requires further investigation.

5.4. VE-cadherin in Clinical Neuroinflammation Disease

Aside from the experimental study of the function of VE-cadherin in BBB dysregulation, our knowledge of the involvement of VE-cadherin in human neuroinflammation disease has also been improved. Ischemic stroke, as a major disease related to BBB dysregulation, has an increased amount of circulating endothelial microparticles in the acute stage [93]. A recent clinical study found that 68 acute ischemic stroke consecutive patients represented a much higher level of VE-cadherin circulating endothelial cells compared to 61 healthy controls (age- and gender-matched) [94]. It is likely that levels of VE-cadherin microparticles might be effectively regarded as a biomarker of cerebral ischemia severity in clinical settings.

In this chronic and progressive CNS disorder, microvascular inflammation and myelination have been identified as central pathophysiological events [95]. Levels of pro-inflammatory cytokines, such as IL-1 and TNF, increased in experimental allergic encephalomyelitis (an animal model of multiple sclerosis), which leads to alterations in BBB permeability [96]. A recent clinical study that comprised 162 patients with chronic coronary artery disease (CAD), 63 patients with non-significant atherosclerosis patients, and 30 healthy controls demonstrated the presence of T-cadherin in 13.3% of healthy controls, in 42.9% of non-significant atherosclerosis patients, and in 34.6% of chronic CAD patients. Interestingly, this study also found that patients with elevated T-cadherin had a lower RH-PAT, suggesting a dependency of T-cadherin and a degree of ED [97]. Patients with type 2 diabetes mellitus and coronary artery disease were also detected with an elevated level of VE-cadherin-positive endothelial microparticles [98].

The relationships between VE-cadherin and other neuroinflammatory diseases, such as Alzheimer's disease or Parkinson disease, have not been well understood. However, increasing evidence demonstrates that VE-cadherin has a instrumental function in neuroinflammatory event progression and BBB dysregulation.

CONCLUSION

Recent studies, described in this review, demonstrated that VE-cadherin-mediated brain vascular endothelial cell adhesion organizes the junctional complex and modulates BBB function. In this regard, VE-cadherin can partially be a biomarker of neuroinflammation disease. On the contrary, VE-cadherin may be a specific molecular target to protect BBB integrity in neurological diseases when junctional proteins dissociate from each other. It also provides us with insight to develop a novel method of modulating BBB function in order to enhance therapeutic delivery into the brain.

ABBREVIATIONS

AJs	=	Adherens Junctions
AP1	=	Activator Protein 1
BBB	=	Blood-Brain Barrier
CAD	=	Coronary Artery Disease
Cdc42	=	Cell Division Control Protein 42 Homolog
CNS	=	Central Nervous System
ECs	=	Endothelial Cells
FAJs	=	Focal Adherens Junctions
FLT-1	=	Fms-like Tyrosine Kinase
IL-1β	=	Interleukin-1β
KDR	=	Kinase Insert Domain-containing Receptor
MMPs	=	Matrix Metalloproteinases
MYD88	=	Myeloid-Differentiation Factor 88
NF-κB	=	Transcription Factors Nuclear Factor-κB
Rac1	=	Ras-related C3 Botulinum Toxin Substrate 1
RIP1	=	Serine/theonine Kinase Receptor-interacting Protein 1
ROS	=	Reactive Oxygen Species
TJs	=	Tight Junctions
TNFR1	=	TNF Receptor 1
TNF-α	=	Tumor Necrosis Fact-α
TRADD	=	Recruits TNFR-associated *via* Death Domain Protein
TRAF2	=	TNFR-associated Factor 2
VEGF	=	Vascular Endothelial Growth Factor
VE-PTP	=	Vascular Endothelial Protein Tyrosine Phosphatase

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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