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REVIEW

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Tight Junction in Blood-Brain Barrier: An Overview of Structure, Regulation, and Regulator Substances

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SUMMARY

Blood-brain barrier (BBB) is a dynamic interference that regulates the nutrition and toxic substance in and out of the central nervous system (CNS), and plays a crucial role in maintaining a stable circumstance of the CNS. Tight junctions among adjacent cells form the basic structure of BBB to limiting paracellular permeability. In the present review, the constituents of tight junction proteins are depicted in detail, together with the regulation of tight junction under stimulation and in pathological conditions. Tight junction modulators are also discussed.

Introduction

The blood-brain barrier (BBB) regulates the flow of essential components into and out of the central nervous system (CNS) and minimizes the influence of toxic compounds and pathogens to the CNS [1,2]. The basic unit of the BBB consists of endothelial cell, astrocyte, pericyte, and the adjacent neurons [3]. The endothelial cells are connected by junction complex, in which tight junction (TJ) plays a significant role.

Except for lipophilic molecules with <8 H-bonds and <400 Da, most molecules could not freely penetrate to the BBB [4]. Substances with high octanol/water partition coefficient could readily penetrate into the CNS [5]. Other molecules, including nutrients with low-partition coefficients, pass through the BBB by paracellular aqueous pathway, transport proteins, receptor-mediated endocytosis, or adsorptive endocytosis [5,6]. Pardridge categorized the transport across the BBB into the following three categories: carrier-mediated transport, active efflux transport, and receptor-mediated transport [4].

In excess of 98% of small molecule drugs do not across the BBB due to the presence of the TJ [7]. With the CNS diseases increasing in the background of longer average life expectancy and most CNS-oriented drugs not being able to enter into the brain, the market for neuropharmaceuticals is potentially one of the largest ones in global pharmaceutical sectors [6]. The current review focuses on the molecular structure and pathological implication of TJ.

Tight Junction

A two-step hypothesis explains the formation of the BBB: endothelial cells initially form leaky vessels; TJ is formed later to create the barrier [8]. Structurally, an intricate combination of transmembrane proteins and cytoplasmic accessory proteins are involved in the TJ formation, and then linked to an actin-based cytoskeleton, allowing TJ to form a seal [9; Figure 1]. Pores between adjacent cells on the seal are important for molecules to pass through TJ, but the basic structure of claudin-based pores is still unknown [10].

Integral Membrane Proteins

Occludin

Occludin is the first identified membrane protein within TJ. Occludin has a molecular weight of 60~65 kDa, and consists of four transmembrane domains, two extracellular domains, and three cytoplasmic domains. Both the N-terminus and C-terminus are intracellular [11]. Occludin forms a homophilically dimer with other

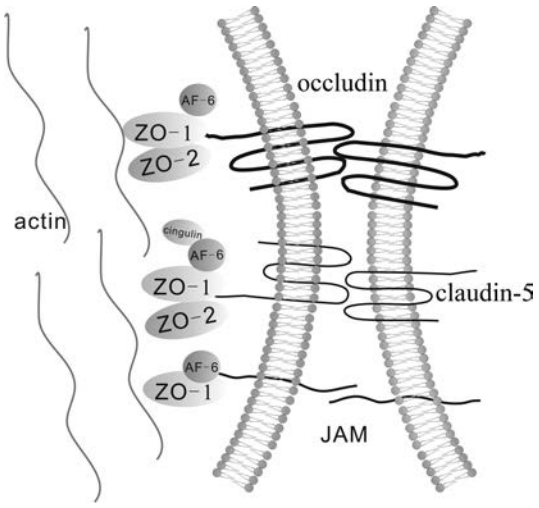

Figure 1 Molecular component of endothelial tight junctions in the BBB.

cells. A uniqiue feature of occludin, relative to other transmembrane proteins is the binding of the 150 amino acid sequence in the C-terminus to F-actin. The C-terminus of occludin could also bind to the GK domain of ZOs, which in turn binds to the cytoskeleton, localizing it to the cell membrane [12].

Occludin could form a TJ-like structure upon transfection into cells that lack TJ [e.g., L-fibroblast; Ref. 12]. Freeze-fracture immunoreplica electron microscopy has also revealed the presence of occludin in TJ fibrils [12]. At the same time, truncation of both the C-terminus and N-terminus of occludin decreases transendothelial electrical resistance, suggesting a key role of occludin in the barrier function of the TJ [11]. Another study reported that occludin-deficient embryonic stem cells have complete TJ as observed in wild-type controls in freeze-fracture replicas [13]. Saitou et al. also failed to observe alteration of barrier function in mice carrying a null mutation in the occludin gene, suggesting that occludin is not required for TJ structural formation [14]. Normal expression and localization of other junctional proteins may compensate for occludin loss [15]. Occludin-deficient mice exhibit complex gross and histological phenotypes, including calcification in the brain, showing the functions of occludin are more complex than just a building block in endothelial cells and other tissues [14].

Claudins

Claudins are a family of 24 proteins with molecular mass ranging from 20 to 24 kDa. Similar to occludin, claudins have four transmembrane domains, two extracellular loops, and a short carboxyl intracellular tail [12]. Claudins are not homologous to occludin in sequence despite of many other similar characteristics.

With the extracellular loops, claudins form the backbone of TJ by forming dimmers and binding homotypically to other claudin molecules in adjacent endothelial cells [16]. With the intracellular loops, they bind to the PDZ domain of zona occludens (ZOs) through their C-terminus.

Claudin-1, -2, -3, -5, -11, and -12 are found in brain endothelial cells [16–18]. Claudin-5 is a hallmark of BBB and plays an essential role in the earliest stage of CNS angiogenesis [2]. Claudin-5 is expressed in the endothelial cells in almost all segments of the brain [19] as well as in blood vessels of the lungs and kidney. Recently, claudin-5 was found to be expressed in epithelial TJ in the human colon cell line HT-29/36 [20]. Nitta et al. found that BBB is permeable to molecules with molecular mass at <800 Da in claudin-5 deficient mice [21]. Consistent with this notion, adrenomedullin could increase the expression of claudin-5 to increase transendothelial resistance and decrease BBB permeability [22]. In addition, an investigation recently found that claudin-5a plays a fundamental role in the early cerebral–ventricular barrier system for the expansion of zebrafish brain ventricular lumen expansion [23].

Junctional Adhesion Molecules

Junctional adhesion molecules (JAMs) belong to the immunoglobulin superfamily, and have a molecular weight at about 40 kDa [24]. JAM includes three structural domains: an extracellular domain with two immunoglobulin-like loops, a single transmembrane domain, and a short intracellular tail [24]. JAM could be categorized into JAM-A (also known as JAM, JAM-1, 106 antigen, and F11R), JAM-B (also known as JAM-2, VE-JAM, hJAM-2 and mJAM-3), JAM-C (also known as JAM-3, hJAM-3 and mJAM-2), and most recently JAM-4 and JAML [25]. All of JAM-A, -B, and -C are found in endothelial cells, with JAM-A highly expressed in the cerebrovessels [17]. Both JAM-A and JAM-C maintain the stability of TJ, and JAM-B maintain the stability of TJ by interacting with JAM-C [26]. Monoclonal antibodies against JAM inhibit re-establishment of barrier function in a transient calcium depletion assay, but do not have any effect to well-formed TJ in confluent monolayers, suggesting that JAM participates in the formation of TJ and is inaccessible in well-formed TJ [12,27]. JAM has been shown to bind to PDZ domain of cytoplasmic proteins AF6 and ZO-1 with its C-terminus in intracellular loops, and extracellularly, JAMs form homophilic interactions with adjacent cells [27].

Cytoplasmic Accessory Proteins

ZO belong to the family of membrane-associated guanylate kinases, and consist of three PDZ domains, one SH3 domain and a guanylyl-kinase domain [28]. ZO proteins play a role in connecting transmembrane proteins to skeleton proteins and interact directly with most of the transmembrane proteins like occludin, claudins and JAM. At the same time, ZO proteins are involved in signal transduction and transcriptional modulation [29].

ZO-1 is a 220 kDa phosphoprotein first found in TJ, and is associated with the C-terminus of claudins by its PDZ-1, JAM by PDZ-2 and -3, and occludin by the GK domain [3,12]. ZO-1 binds to the actin cytoskeleton through its C-terminus, serving as a bridge between transmembrane proteins and skeleton proteins. This interaction is important to the stability and function of TJ; dissociation of ZO-1 from the junctional complex often leads to increased permeability of BBB [3]. Once localized to the transmembrane

proteins complex, ZO-1 becomes insoluble in nonionic detergents, showing strong association with skeleton actin [30]. In Eph4 cells lacking ZO proteins, the formation of TJ is completely disrupted, with claudins failing to polymerize at TJ in these cells, showing that the SH3/GUK domains of ZO proteins play a critical role for claudin polymerization [31].

ZO-2 is a 160 kDa phosphoprotein that shares sequence homology with ZO-1. ZO-2 functions redundantly with ZO-1, so it may replace ZO-1 and facilitate formation of a morphologically competent TJ [32]. More recently, an investigation found that ZO-2 deficient mice suffered early embryonic lethality, with a decreased proliferation at embryonic day 6.5 and increased apoptosis at embryonic day 7.5. This finding suggests that ZO-2 is required for mouse embryonic development, and cannot be compensated by ZO-1 and ZO-3 [33].

ZO-3 is a 130 kDa phosphoprotein that shares homologous sequence and domain organization with ZO-1, and could coimmunoprecipitate with the ZO-1/ZO-2 complex [12]. ZO-3 has not been found in the BBB up to now [34].

Other Cytoplasmic Accessory Proteins

Other accessory proteins in BBB include cingulin, AF-6 (afadin), and 7H6 antigen [3].

Cingulin is a 140 kDa protein, interacting with other TJ proteins ZO-2, AF-6, JAM, skeleton protein F-actin, and other cingulin molecules [12,35]. Embryoid bodies with deficient cingulin show normal TJ structure and normal localization of ZO-1, occludin, and claudin-6. The transcript levels for claudin-2, -6, -7 and occludin increased. The study suggests that lacking of cingulin does not disrupt TJ formation but alter gene expression and TJ protein levels [36]. Interestingly, inducible overexpression of cingulin in stably transfected MDCK cells showed no significant affection in organization and function of TJ, TJ protein levels, and gene expression, indicating that modulation of cellular functions by cingulin occurs under physiological levels [37].

AF-6 is a 205 kDa protein that contains one PDZ domain, two Ras binding domains and regions of homology with kinesin and myosin V [12]. AF-6 is associated with ZO-1 through two Ras-associating domains, and could be inhibited by Ras activation, indicating that disruption of the ZO-1/AF-6 complex may be critical in the modulation of TJ [3]. AF-6 deficient mice are developmental retarded at stages during and after gastrulation, indicating that AF-6 performs an important role in TJ [38].

7H6 antigen is a 155 kDa protein with a putative ATPase domain for reversibly dissociating from the TJ under conditions of ATP depletion [3,12]. Upregulation of 7H6 antigen with dibutyryl-cAMP or all-trans-retinoic acid in endothelial cells could enhance barrier function, suggesting that 7H6 antigen plays a significant role in the regulation of paracellular barrier function [39].

BBB Changes in Pathological Conditions

Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease with amyloid-β accumulation on blood vessels and parenchyma neurofibrillar tangling in the brain [40]. Clinical features include dementia, neuropsychiatric symptoms, seizures, and epilepsy [41]. In vitro experiments demonstrated that amyloid-β deposition downregulates the mRNA and protein level of ZO-1 and occludin, and disturbs organization of claudin-5, suggesting that AD affects the function of BBB by altering the expression and location of TJ proteins [42]. MRI studies in human subjects indicated that BBB permeability is present at an early phase of AD [43]. A study involving 157 AD patients found that the BBB is dysfunctional in part of the patients and the dysfunction may accelerate the progress of AD by affecting the clearance of amyloid-β in turn [44].

Ischemic and Hypoxia Insults

Stroke, mostly ischemic, is the second most common cause of death and disability in developed countries [45,46]. Many complex pathological conditions come out under the progression of ischemic stroke, including neuronal depolarization (excitotoxic glutamate efflux), intracellular calcium increase, metabolic function loss, oxidative stress, and activation of inflammation [17,47,48]. All these changes could have major influences on the BBB. Oxygen could alter the location, decrease the protein expression of claudin-5 in bend.3 cells, and disrupts BBB [49]. Exposure to 6% O_2 increases TJ proteins claudin-5, occludin, and ZO-1 and disrupted organization at endothelial cell margins [50]. In a study of ischemia-reperfusion, the mRNA and protein levels of claudin-5, occludin, and ZO-1 significantly decreased, together with an increased permeability of BBB [17]. The opposite results in the expression levels of TJ proteins may be explained by whether to reperfuse after ischemia. When Wistar rats were exposed to hypobaric hypoxia, the permeation of BBB increased significantly, showing the function of BBB could be damaged by acute hypoxia [51].

Activation of matrix metalloproteinases (MMPs) after ischemic stroke could decrease the expression of claudin-5 and occludin, and increase BBB permeability [52]. MMPs inhibitor could partly reverse the degradation of the TJ proteins [53]. Normobaric hyperoxia treatment in the early stage of BBB disruption following ischemic stroke could inhibit MMP-9-mediated occludin degradation and attenuate BBB disruption [54].

Inflammation

The permeability of BBB could be altered by the chronic inflammatory pain. Chronic inflammatory pain induced by injection of complete Freund's adjuvant into the plantar hindpaw disrupts the integrity of BBB and is associated with decreasing in the expression of occludin [55]. Injection of λ-carrageenan into the plantat hindpaw could cause peripheral inflammatory pain, resulting in the increase of BBB permeability, promoted by the disruption of occludin oligomeric assemblies [56]. Patients with rheumatoid arthritis have increased morbidity and mortality due to cerebrovascular diseases [57]. Based on a large observational study in Japan, cerebrovascular disease is the third major cause of death in rheumatoid arthritis [58]. A recent study found that

rheumatoid arthritis could increase permeability of BBB, together with a decrease of occludin but not ZO-1 [57].

Multiple sclerosis (MS) is considered as a neuroinflammatory disease [3,59], with myelin sheath damage as the major pathological change. BBB abnormality could be detected before MRI changes [3], at both focal and diffuse level in MS patients with postmortem MRI in another study [60]. Kirk et al. detected the expression of ZO-1 in focal, diffused zone and the control showed similar results. Forty-two percent vessel segments in focal area, 13% in normal-appearing white matter are abnormal versus 3.7% in normal controls, suggesting that the progressing breakdown of the BBB is accompanied by the progression of MS [61]. Even though the BBB is disrupted in MS patients, tool compounds including lipophilic transcellular drugs, hydrophilic paracellular compounds and P-gp substrates, could not penetrate though the BBB [62].

Diabetes Mellitus

Diabetes mellitus is associated with changes in the barrier function of cerebral microvessels. These changes may contribute to the CNS complications of diabetes mellitus [63]. Recently, clinical evidence suggests that diabetes-induced changes in the BBB lead to increased incidences of many CNS diseases, such as hemorrhage, vascular dementia, and may be a predisposing factor of AD [64]. The expression of TJ proteins could be altered by the pathology of diabetes. Streptozotocin-induced diabetes could decrease the expression of occludin in cerebral microvasculature [65]. In addition, treatment of diabetes with insulin attenuates BBB disruption in the first few weeks, whereas microvascular damage is irreversible even when hyperglycemia is controlled as diabetes progressed [66]. More recently, Hawkins et al. adds that the expression of ZO-1 decreases in streptozotocin-induced diabetes, and the increase of MMPs activation together with hyperglycaemia may be the underlying causes of hyperpermeability of BBB in diabetes mellitus [67]. Animals with diabetes mellitus are more vulnerable to nanoparticle-induced cerebrovascular reactions in the brain and neuronal damage [68]. In addition, high-energy diet increases the permeability of BBB, with a decrease in mRNA level of claudin-5 and claudin-12 [69]. Interestingly, the most vulnerable area of the brain is hippocampus, which may explain the relationship of high-energy diet with AD [69].

HIV

Human immunodeficiency virus type 1 (HIV-1) can cause CNS complications, such as HIV-related encephalitis and HIV-associated dementia [70]. HIV infection can alter the structure and function of TJ, decrease the expression and alter the distribution of claudin-1, ZO-2, claudin-5 and JAM-A [71–73], yet the expression of occludin and ZO-1 remains unchanged [70,71]. Rho/RhoK activation may be an underlying cause of BBB impairment during HIV-related encephalitis [74]. Mitogen-activated protein kinase 1/2, vascular endothelial growth factor receptor type 2, phosphatidylinositol-3 kinase, nuclear factor-kappa B and signal transducers and activators of transcription may involve in Tat-induced alteration of TJ proteins level [75,76]. HIV Tat protein injection could upregulate expression of cyclooxygenase-2 and decrease expression of TJ proteins, whereas cyclooxygenase-2 inhibition partly attenuates the downregulation of TJ proteins, showing that cyclooxygenase-2 plays limited roles in HIV-related BBB dysfunction [77]. Recently, a study showed that stimulation of PPAR activity or overexpression of PPAR could attenuate HIV-induced alterations of TJ proteins and disruption of barrier function of BBB [73].

Drug Abuse

Methamphetamine (METH) and cocaine are powerful psychostimulant drugs used illegally throughout the world. Acute METH intoxication may lead to disruption of the BBB and acute brain edema [78,79]. METH has shown to alter permeability of BBB especially in hippocampus [80]. METH administration decreases the expression level of claudin-5, occludin and ZO-1, together with an increased activation of MMP-9, suggesting that the effects of METH on BBB function can be explained by alterations on TJ proteins and MMP-9 [80]. Exposure to METH enhances reactive oxidative stress in brain microvascular endothelial cells (BMEC), which then activates myosin light chain kinase and decreases TJ proteins levels [81]. Effects of cocaine on BBB are generally similar to METH. Exposure to cocaine can result in increasing permeability of endothelial barrier, together with an increased expression of platelet-derived growth factor. Platelet-derived growth factor neutralizing antibody could abrogate this effect, indicating an essential role of platelet-derived growth factor [82].

Many HIV patients are addicted to neurotoxic drugs such as METH, morphine, and cocaine, which may worsen the HIV-related neurological impairments in turn. When treated with METH and gp120 (an envelope glycoprotein of HIV-1), the expression of ZO-1, claudin-3, claudin-5 and JAM-2 in BMEC decrease significantly, with an increase in the expression of occludin. Treatment with gp120 alone decreases the expression of ZO-1, claudin-3, JAM-2, and has no effect on claudin-5 and occludin [83]. METH modulates TJ proteins expression and decreases barrier function of BBB via Rho-A activation [83]. Morphine treatment BMEC could decrease the expression of TJ proteins ZO-1 and occludin significantly, while increasing the expression of JAM-2. It compromises barrier function in vitro, possibly through activation of pro-inflammatory cytokines, intracellular calcium release and activation of myosin light chain kinase [84]. Cocaine has a direct effect on TJ proteins in BMEC. An investigation found that exposed to cocaine may downregulate the expression of ZO-1 protein and upregulate the CCL2/CCR2, which plays a crucial role in the progression of HIV-1 neuropathogenesis [85]. Exposing BMEC to cocaine may upregulate MMP, increase expression of TNF-α, thereby suggesting that cocaine causes membrane permeability and endothelial transmigration of HIV-infected dendritic cell [86].

Regulation of TJ in BBB

Environmental Regulation

Now, more and more investigations recognize the relationship between the integrity of BBB and environmental regulations. The

permeability of BBB could be affected by the physical and chemical changes in milieu. A finding demonstrates the importance of the shear stress in the formation of BBB, which could upregulate the expression of ZO-1 and occludin, enhancing bovine brain microvascular endothelial barrier function [87]. An interesting investigation found the impact of culture pH and buffer concentration on the paracellular tightness in vitro. Co-culture with astrocytes increases barrier function of bovine brain capillary endothelial cells independent of the type of buffer detected by transendothelial electrical resistance [88]. Another research focused on the toxicity of manufactured aluminum oxide nanoparticles, which have been widely found in the environment. They found that the treatment with nanoalumina could markedly reduce the viability of BMEC and decrease the integrity of claudin-5 and occludin. And the alterations could be prevented by glutathione [89]. It shows that the possible mechanisms may exist in the oxidation produced by the clearance of the BMEC. Another study took temperature into account. Barrier function is compromised by brief heat shock, and more time is needed to recover under higher temperature. Repeated application of heat treatment could produce a reinforced barrier in the BMEC [90].

In another way, people make great efforts to enhance the drug targeted to CNS through BBB. Sheikov et al. found that ultrasound burst in combination with gas contrast agent could redistribute and reduce the expression of occludin, claudin-5, and ZO-1, but not claudin-1. After sonication, the dysfunction of BBB could last for as long as 4 h [91]. Recently, Fan et al. found that the expression of ZO-1, occludin, and claudin-5 decreased most significantly under low-frequency ultrasound irradiation at 2 h, and gradually returned to the original level at 24 h, together with the permeability of BBB increasing [92]. Exposed to electromagnetic pulse could also increase the permeability of BBB and decrease levels as well as alter localization of ZO-1 [93]. The aforementioned methods could be used to increase the permeability of BBB temporarily to deliver drugs into the CNS more efficiently.

Hormone Level

Because cerebral vasculature is an important target of estrogen [94], many investigations have been performed to confirm that the BBB is more accessible in older females (reproductive senescent) than in younger ones [95]. Hormonal decline, marking reproductive senescence, leads to increased permeability of the BBB, which is further exacerbated by estrogen treatment in special regions [96]. Poor junctional localization of claudin-5 in hippocampal microvessels exists in the reproductive senescent females than in the younger ones, indicating that dysregulation of claudin-5 may associate with ovarian aging [96]. Dissimilarly, Sandoval et al. held a different view that under the treatment of 17β- estradiol, the expression of claudin-5 varies in the younger animals and the middle-aged ones, but the functional paracellular permeability measured via the in situ perfusion of [14C]-sucrose shows no change between the two groups [97].

Glucocorticoids are widely used as a therapeutic strategy for the disruption of BBB in neuroinflammatory conditions. A study found that occludin is a direct target of glucocorticoids. Hydrocortisone at physiological concentrations induced upregulation of occludin, together with an enhancement of barrier function of BBB [98], possibly by preventing endothelial barrier breakdown in response to TNFα stimulation [99]. Maternal exposure to glucocorticoids could increase the expression of claudin-1, claudin-5, ZO-2, together with the decrease in BBB permeability in the fetus [100].

TJs' Regulator Substances Targeted at the BBB

Zonula Occludens Toxin

Zonula occludens toxin (Zot) is a 45 kDa protein. Its receptor in the human brain may be a target for Zot and other analogues mimic the functional components to modulate the permeability of the BBB [101]. Studies already demonstrated that Zot could enhance penetration of drugs with different molecular weight and low bioavailability across the bovine BMEC in a concentration dependant manner [102]. More importantly, the effect of Zot is reversible and nontoxic [103]. DeltaG, a biologically active fragment of Zot with a 12kDa molecular weight, was identified in 2001. DeltaG administrated via an intracarotid injection could increase the hydrophilic and lipophilic drugs across the BBB significantly [104].

Other Modulators Targeted at the BBB

There are many other TJ modulators targeted at the BBB, such as calcium chelators, surfactants, cationic polymers, cyclodextrins, and hyperosmotic solutions [105]. However, none of these TJ modulators is specific towards the TJ in BBB. Many of these molecules (e.g., surfactants) enhance the transportion of drugs by disrupting the structure of the lipid bilayer [103]. The cytotoxic characteristics of the TJ modulators limit their further study.

Conclusions

The basic structure of BBB was demonstrated over 100 years ago. However, the complex factors in physiological and pathological conditions still need investigations. The disruption of TJ may increase permeability of BBB, resulting in serious diseases in the CNS. On the other hand, pathological state in CNS is always accompanied by the downregulation of TJ proteins and dysfunction in BBB. Studies in the BBB will revive with the increasing morbidity in the brain.

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Conflict of Interest

The authors declare no conflict of interest.

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