Occludin regulation of blood–brain barrier and potential therapeutic target in ischemic stroke

Shuhua Yuan, Ke Jian Liu, Zhifeng Qi

Abstract:
Occludin is a key structural component of the blood–brain barrier (BBB) that has recently become an important focus of research in BBB damages. Many studies have demonstrated that occludin could regulate the integrity and permeability of the BBB. The function of BBB depends on the level of occludin protein expression in brain endothelial cells. Moreover, occludin may serve as a potential biomarker for hemorrhage transformation after acute ischemic stroke. In this review, we summarize the role of occludin in BBB integrity and the regulatory mechanisms of occludin in the permeability of BBB after ischemic stroke. Multiple factors have been found to regulate occludin protein functions in maintaining BBB permeability, such as Matrix metalloproteinas-mediated cleavage, phosphorylation, ubiquitination, and related inflammatory factors. In addition, various signaling pathways participate in regulating the occludin expression, including nuclear factor-kappa B, mitogen-activated protein kinase, protein kinase c, RhoK, and ERK1/2. Emerging therapeutic interventions for ischemic stroke targeting occludin are described, including normobaric hyperoxia, Chinese medicine, chemical drugs, genes, steroid hormones, small molecular peptides, and other therapies. Since occludin has been shown to play a critical role in regulating BBB integrity, further preclinical studies will help evaluate and validate occludin as a viable therapeutic target for ischemic stroke.

Keywords:
Blood-brain barrier, ischemic stroke, occludin

Introduction
Ischemic stroke is characterized by the occlusion of the cerebral artery or arteries supplying the brain tissues, resulting in neuronal death within minutes in the corresponding brain regions.[1,2] Accumulating evidence support that blood-brain barrier (BBB) damage is an early event after ischemic stroke, leading to pathophysiological damages in the brain. BBB damage further results in progressive neuronal death and cerebral edema, even intracerebral hemorrhage transformation after ischemic stroke.[3] Therefore, preserving BBB integrity is of critical importance in designing treatments for ischemic stroke.

Occludin, as a member of tight junction proteins, is a key structural component of the BBB,[4] and occludin degradation can be seen in the course of ischemic stroke, leading to the disruption of BBB.[5-8] In this review, we summarize the role of occludin in maintaining BBB integrity and the molecular mechanisms of occludin interruption, leading to alteration of the BBB permeability after ischemic stroke.

Blood–Brain Barrier and Tight Junctions

Many previous studies demonstrated that a physical barrier, which was subsequently identified as BBB, exists between the central nervous system and the peripheral circulation to prevent the toxic and harmful substances from invading brain tissue.[9,10]
BBB is mainly composed of brain endothelial cells, tight junctions, astrocyte end-feet, pericytes, microglial cells around blood vessels, and basement membrane.\[^{11}\] BBB prevents the brain from neurotoxins, neurotransmitters, and macromolecules, and passively transporting water-soluble nutrients or metabolites and gases, maintaining the balance of the microenvironment in the brain.\[^{12}\]

Compared to epithelial tight junctions, endothelial tight junctions in BBB are highly special in molecular structure with more restrictive paracellular diffusion barrier, lower transcytotic vesicles and more sensitive to the microenvironment.\[^{13}\] Tight junctions between endothelial cells are made up of zonula occludens (ZO) and transmembrane proteins, such as occludin, claudins, junctional adhesion molecules, and tricellulin, linked to the cytoskeleton and cytoplasmic scaffold proteins.\[^{14}\] The changes of the sealing proteins in conformation or modified adjusting have a direct effect on the state of tight junctions and further, affect the permeability of BBB.\[^{15}\]

Recently, occludin has been found to play a critical role in BBB integrity. Many studies showed that occludin degradation or abnormal occludin could be found to increase BBB permeability in various central nervous system diseases,\[^{16}\] especially in ischemic stroke, disturbing the stability and normal function of the brain. Therefore, it is essential to further understand the function and regulation mechanism of occludin.

### The Structure of Occludin and Blood-Brain Barrier

Furuse et al. originally extracted the proteins from poultry tissues and identified it as an integral membrane protein in epithelial and endothelial cells.\[^{17}\] Occludin is indispensable for barrier integrity in diverse endothelial cell models,\[^{18}\] but some studies reported that other tight junctions in epithelial cells were able to maintain morphological integrity in occludin deficient models,\[^{19}\] suggesting that occludin is prone to protecting barrier function rather than assembly in BBB.

The barrier function of occludin depends on its special structure domains. Occludin is a 65 kDa integral membrane protein with 502 amino-acid residues, which includes two extracellular loops, one intracellular loop, and its carboxyl and amino-terminals oriented toward the cytoplasm. The first extracellular loop (ECL1) of occludin has a very high content of tyrosine and glycine residues (about 55aa). The tyrosine residues are involved in forming hydrophobic interactions and H-bonds, while glycine residues provide flexibility. The second extracellular loop (ECL2, about 45aa) is rich in tyrosine residues and contains two cysteines to form disulfide bridges in the oxidizing environment, which is sensitive to hypoxia and occurs homo-oligomerization.\[^{20}\] Compared to ECL1, ECL2 serves as the main binding domain, which interacts with other tight junctions and regulates the function of other tight junctions. In addition, C-terminal cytoplasmic of occludin (about 259aa) is rich in serine, threonine, and tyrosine residues and directly connects to ZO-1 and actin cytoskeleton.\[^{21}\] It contains the main binding sites for regulatory molecules, such as connexin 26 or different kinases.\[^{22}\] Both external loops as well as the transmembrane and the C-terminal cytoplasmic domains of occludin, are important for the regulation of paracellular permeability between adjacent cells.

### The Regulation of Occludin and Blood–Brain Barrier Integrity

At present, multiple factors have been found to regulate occludin functions on BBB permeability,\[^{23}\] such as matrix metalloproteinases (MMPs)-dependent degradation, phosphorylation, ubiquitination, and other cytokines.

#### Matrix metalloproteinase-dependent occludin degradation

MMPs are secreted as zymogens and cleaved to be active. In vivo and in vitro evidence show that the levels of active MMP-2 and MMP-9, which are extremely low in normal brain tissue, mediate occludin degradation in pathological conditions.

BBB was damaged via vascular endothelial growth factor (VEGF)-mediated MMP-9 activation in a hypoxia mouse model, leading to the reduction of occludin expression, but there was no significant change in the expression of claudins and ZO-1.\[^{24}\] Besides, the activity of MMP-9 could be suppressed by the inhibition of the nuclear factor-kappa B (NF-kB) pathway in the transient middle cerebral artery occlusion (MCAO) mouse model, and the expression of occludin, JAM-A and ZO-1 proteins in brain tissue was elevated, which helps to protect BBB integrity.\[^{25}\]

In addition, the expression of occludin significantly decreased by the MMP-2/MMP-9 activation in brain microvascular endothelial cells (BMECs) in an OGD/R-injury neurovascular unit model, which involved the mitogen-activated protein kinase (MAPK) pathways.\[^{26}\] Accumulating evidence shows that activation of MAPK signaling pathways contributes to BBB damage,\[^{27}\] leading to MMP-9 expression increase and reducing the level of occludin, ZO-1, and claudin-5 in BMECs.

#### Occludin Phosphorylation

Occludin phosphorylation has been identified as an important regulatory mechanism in regulating BBB integrity. Numerous studies showed that the
BBB permeability is related to the status of occludin phosphorylation at serine/threonine or tyrosine.\textsuperscript{[38-31]} Occludin has multiple phosphorylation sites, among which the Ser-507, Thr-382, Ser-490, and Ser-338 are classical phosphorylation sites. The state of occludin phosphorylation has different effects on BBB permeability, depending on the phosphorylation types of occludin or diverse signaling pathways.

Some studies have described the regulation of occludin by protein kinase c (PKC). An in vitro study in LLC-PK1 cells showed that activation of PKC with 12-O-tetradecanoylphorbol-13-acetate (tissue plasminogen activator [TPA], a PKC activator) reduced threonine phosphorylation of occludin protein, resulting in increased paracellular permeability.\textsuperscript{[32]} However, TPA improved barrier function and upregulated the expression of occludin protein via activation of PKC.\textsuperscript{[33]} Further, inhibition or knockdown of PKC induced dephosphorylation of occludin on threonine residues (T403 and T404) in Caco-2 and MDCK cell monolayers.\textsuperscript{[34]} These studies indicated that PKC may have opposite effects on occludin protein regulation in the signaling pathway under different conditions.\textsuperscript{[35]}

In addition, VEGF-induced occludin phosphorylation increased BBB permeability.\textsuperscript{[36]} While inhibition of VEGF-induced PKC-β activation reduced the barrier permeability by preventing occludin phosphorylation at Ser-490.\textsuperscript{[37]} Moreover, an in vivo study reported that Ser-490 was a special key site for regulating the expression of occludin or diverse signaling pathways.

Further, Walsh et al. reported that vascular endothelial cadherin could transmit physiological shear signals to occludin via the Tiam1/Rac1 signaling pathway in brain microvascular endothelial cells (BMECs) model, resulting in Tyr-occludin dephosphorylation and reduced BMECs permeability.\textsuperscript{[39]} Moreover, another study demonstrated that cyclic strain (5% strain, 60 cycles/min, 24 h) induced the expression of occludin/ZO-1 proteins upregulation in endothelial cells, with the tyrosine phosphorylation of occludin reduced and the serine/threonine phosphorylation of ZO-1 increased.\textsuperscript{[40]} Besides, there are many neurotransmitters involved in tyrosine phosphorylation of occludin. Glutamate increased tyrosine phosphorylation and decreased threonine phosphorylation of occludin in brain microvascular endothelial cells through N-methyl-D-aspartate or alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate/kainite receptors, resulting in disruption of the BBB.\textsuperscript{[41]} Treatment of microvascular endothelial cells with protein phosphatase type 2A (PP2A) showed that PP2A dephosphorylated the tyrosine residue of occludin, leading to the reduction of occludin expression. Moreover, inhibiting the activity of PP2A maintained the BBB integrity via hyperphosphorylation of tyrosine residues of occluding.\textsuperscript{[42]} When monolayer microvascular endothelial cells were exposed to septic insults, PP2A reduced serine and threonine phosphorylation of occludin, leading to increased monolayer permeability.

**Occludin ubiquitination**

Ubiquitin is a heat-stable 76-amino acid protein, which was found in eukaryotic cells and coupled to lysine residues in an ATP-dependent manner as a monomer or polymer, guiding the protein to the proteasome degradation pathway.\textsuperscript{[43]} Ubiquitination is an important mechanism for regulating the function of target proteins by modifying their intracellular transport and degradation within endothelial tight junctions.

Lui and Lee et al. reported that occludin degradation was associated with Itch and UBC-4 (an ubiquitin-conjugating enzyme), which was mediated by dibutyryl-cAMP pathway, resulting in occludin ubiquitination to disrupt tight junctions in blood and testosterone barrier cells.\textsuperscript{[44]} Furthermore, Nedd4-2, a member of E3 ubiquitin ligase, was co-immunoprecipitated with occludin through interacting on the conserved PY motif in the C-terminal of occludin, suggesting that Nedd4-2 ubiquitinated occludin and induced occludin degradation.\textsuperscript{[45]} Moreover, silencing Nedd4-2 upregulated the expression of occludin and reduced paracellular permeability in mpk-CCDc14 cells. Recently, Murakami et al. demonstrated that occludin ubiquitination regulates endothelial permeability in bovine retinal endothelial cell models via the VEGF-mediated pathway.\textsuperscript{[46]} It was shown that phosphorylation of occludin at Ser-490 is necessary for its ubiquitination after VEGF treatment. Finally, occludin ubiquitination induces the endocytosis of related tight junction proteins (such as claudin-5 and ZO-1), which ultimately lead to the destruction of the BBB.\textsuperscript{[47]}

**The interaction with cytokines**

Cytokines are involved in a multitude of molecular mechanisms in regulating occludin expression and mediating changes in BBB integrity.

**Tumor necrosis factor-α**

Tumor necrosis factor-α (TNF-α) is known to induce changes in endothelial cell morphology and permeability, but the mechanisms have not been extensively characterized.\textsuperscript{[48]} Ni et al. demonstrated that TNF-α induced upward-band shift of occludin (phosphorylation) in the human cerebral endothelial cell line (hCMEC/D3) by transient stimulation of p38MAPK and ERK1/2 pathway, increasing cerebral endothelial cells permeability and
leading to BBB disruption.[48] In renal endothelial cells, TNF-α-induced barrier dysfunction or the reduction of occludin expression is crucially dependent on the Rho/MLCK signaling pathway.[50] TNF-α also downregulated occludin expression by activating HIF-1α/VEGF/VEGFR-2/ERK signaling pathway, which was inhibited by propofol.[51] In addition, platelet endothelial cell adhesion molecule-1 (PECAM-1) expression is involved in maintaining barrier function in endothelial cells. Combined treatment of TNF-α and dengue virus decreased barrier function, altered distribution of PECAM-1, and lower level of occludin protein in human endothelial cells.[52] Furthermore, NADPH in human astrocytes enhanced TNF-α-induced barrier dysfunction, accompanying with the activation of MMPs and reduction in occludin expression.[53] Moreover, decreasing NADPH activation by inhibiting TNF-α improves the barrier function through upregulating the expression of occludin proteins.[54] These studies suggest that TNF-α degrades occludin and promotes BBB damage by multiple signaling pathways.

**Interleukin-1β**

As one of the pro-inflammatory cytokines, interleukin-1β (IL-1β) plays an important role in regulating the expression of occludin in inflammation.[55] Toshiaki Abe et al. found in cultured human retinal pigment epithelial cell line (ARPE-19) that IL-1β downregulated the expression of occludin but upregulated claudin-1, compared with the control medium,[56] suggesting that IL-1β induces the loss of occludin proteins. Some studies explored the mechanisms of interaction between IL-1β and occludin. The activation of ATP/P2 X 7R (a unique purinergic receptor) signaling pathway induced degradation of occludin, and ZO-1 proteins were associated with the release of IL-1β and enhancement of the MMP-9 activity in the human model of BBB in vitro.[57] Besides, the NF-kB pathway is essential for IL-1β to induce the redistribution of occludin and ZO-1 proteins, resulting in BBB disruption in cultured human corneal epithelial cells.[58] This was consistent with a recent study that Mdvi-1 (a selective dynamin-related protein-1 inhibitor) alleviated the brain edema after subarachnoid hemorrhage to inhibit NF-kB dependent inflammation via suppressing occludin degradation and IL-1β release.[59] Further, IL-1β-induced MMP-9 expression and activation in pericytes suppressed the expression of occludin proteins in the BBB model and led to increased BBB permeability, which was regulated by the NOTCH3/NF-kB signaling pathway.[60] Inhibiting IL-1β and montelukast (leukotriene receptor type 1 antagonist) enhanced the expression of occludin and ZO-1 proteins and protected against BBB disruption.[61,62] However, another study reported that IL-1β increased the expression of claudin-1, but there was no significant change in the expression of occludin in cultured HaCaT keratinocytes. The different effects may be associated with the stage of skin healing, but the underlying mechanism is still not clear.[63]

**Interferon-γ**

Interferon (IFN-γ), secreted from activated T and natural killer cells, not only plays immunomodulatory roles in inflammation but also modifies endothelial barrier function.[64] Studies showed that IFN-γ maintains BBB integrity by enhancing the expression of occludin and ZO-1 in the experimental autoimmune encephalomyelitis mice model.[65] However, other studies reported that IFN-γ has the opposite effect on BBB integrity.[66] For example, occludin expression significantly decreased after IFN-γ treatment in human umbilical vein endothelial cell layers,[67] while occludin protein disassembly was observed when exposed to IFN-γ in BBB transwell model in vitro.[68] In addition, the study demonstrated that the extent of BBB damage was related to the concentration of cytokines such as IFN-γ in a dose-dependent manner via JNK signaling pathways.[69] In summary, these studies showed that IFN-γ regulates BBB function through interacting with occludin in diverse experimental models.

**Hepatocyte growth factor**

Hepatocyte growth factor (HGF) is a multifunctional cytokine, including mitogenic, motogenic, morphogenic, angiogenic, and anti-apoptotic activities in diverse types of cells,[70] which help alleviate ischemia-induced injuries via anti-apoptotic and angiogenic activities.[71,72] One study demonstrated that human recombinant HGF relieved the extent of BBB disruption in a microsphere-induced cerebral embolism rat model, and mitigated the reduction of occludin expression.[73] However, there was an opposite finding that treatment of human microvascular endothelial cells with HGF decreased transendothelial cell resistance as well as occludin expression, and increased paracellular permeability.[74] In general, HGF plays its role in regulating the function of tight junctions by altering the phosphorylation state of occludin and it does not change the phosphorylation of ZO-1.[75] These studies indicate that HGF can regulate the expression level of occludin, leading to changes in the function of tight junctions, but the mechanism of HGF in regulating phosphorylation of occludin is still unknown. Further investigations are needed to clarify the mechanism.

**Occludin and Ischemic Stroke**

Ischemic stroke is one of the most common diseases with high mortality and disability, which accounts for around three-fourths of all strokes.[76] Many studies have demonstrated that disruption of BBB is an early event after ischemic stroke, which may develop into
hemorrhagic transformation at later time points following ischemia-reperfusion.\textsuperscript{[77-79]} Occludin serves as one of the key structural tight junction proteins for BBB integrity. Animal and human studies have indicated that occludin degradation is frequently seen in ischemic stroke and contributes to BBB injury.\textsuperscript{[5,8]} Therefore, we focus our attention here on occludin degradation and regulation in ischemic stroke.

**Occludin degradation into fragments by matrix metalloproteinases in ischemic stroke**

Our previous studies demonstrated that occludin degradation and claudin-5 redistribution is seen in isolated ischemic cerebral microvessels from MCAO rat models, causing the disruption of BBB integrity.\textsuperscript{[7]} In other studies, increased MMP-2/9 activation in the ischemic brain contributes to BBB disruption through enhanced occludin degradation.\textsuperscript{[8,60]} Further, Pan et al. demonstrated that the level of blood occludin fragments increases proportionately to the extent of BBB damage at the early stage of cerebral ischemia in an MCAO rat model,\textsuperscript{[81]} and most importantly, the worst BBB damage occurs at 4.5-h after stroke onset, coincident with the peak level of occludin fragments in the peripheral blood. These studies indicate that blood occludin may be a biomarker of BBB damage, thus could serve as a potential predictor of hemorrhagic transformation in ischemic stroke patients.\textsuperscript{[82]} The level of serum occludin is detected by enzyme-linked immunosorbent assay at present. However, this method could not distinguish the fragments of occludin from the full-length proteins in the serum. Further studies are required for developing specific method to improve sensitivity and specificity.

**Occludin regulation in ischemic stroke**

Owing to BBB damages playing a pivotal role in ischemic stroke, there are many studies on occludin regulation to protect BBB in cerebral ischemic animals or patients.

**Normobaric hyperoxia treatment**

As tissue hypoxia is a critical event in the pathophysiology of ischemic stroke, supplement of oxygen to ischemic tissue has long been thought of as a logical stroke treatment strategy.\textsuperscript{[83]} Treatment with normobaric hyperoxia (NBO, 100% oxygen) increases ischemic tissue oxygenation by maintaining the penumbral PO\textsubscript{2}, level close to the pre-ischemic level.\textsuperscript{[84]} Early NBO treatment is neuroprotective via delaying the progression of ischemic brain tissue necrosis, which is equivalent to saving time and expanding the window of opportunity for reperfusion therapies.\textsuperscript{[85,86]} Importantly, combination treatment of NBO and recombinant tPA in the MCAO rat model could lessen BBB disruption and reduce hemorrhagic transformation, compared with rats which underwent delayed tPA treatment at 5 or 7 h postischemia.\textsuperscript{[87]} These studies indicate that NBO treatment after ischemia stroke onset helps to rescue the ischemic penumbra and microvessels, and has a great potential for serving as a promising adjuvant therapy to extend time window of tPA thrombolysis or thrombectomy for ischemic stroke.\textsuperscript{[88]}

Our previous studies also investigated the mechanisms of NBO on BBB protection. NBO treatment played a vital role in slowing the progression of BBB disruption via inhibiting the activity of MMPs and the consequent occludin degradation.\textsuperscript{[5]} In addition, the study of Liu et al. suggested that NBO treatment protects the BBB against ischemic damages by reducing MMP-9 activity and enhancing the expression level of occludin proteins in microvessels, which was inhibited by gp91\textsuperscript{phox} (also called Nox2) in an MCAO mouse model.\textsuperscript{[89]} In addition to combined treatment with NBO and tPA, there was another report that NBO plus minocycline could effectively reduce the extent of ischemic brain injury and protect BBB due to inhibition of MMP-2/9 activity or gp91\textsuperscript{phox} (also called Nox2) in ischemic brain tissue and alleviating the extent of BBB damage in ischemic stroke, which makes NBO a promising auxiliary approach to expand the narrow time window of reperfusion therapies for ischemic stroke.\textsuperscript{[90]} Our previous study has shown that NBO can be applied in ischemia/reperfusion injury patients, especially for tPA thrombolysis in ischemic stroke in the clinic.

**Chinese medicines**

A lot of traditional Chinese medicines have been reported to reduce the ischemic brain damages by regulating BBB permeability with various therapeutic mechanisms. In the oxygen-glucose deprivation/recovery (OGD/R)-injured neurovascular unit model, Zhao et al. demonstrated that cryptotanshinone inhibited MAPK signaling pathway to ameliorate neuron apoptosis and elevated the expression of occludin protein through down-regulation of MMP-9 expression.\textsuperscript{[26]} Liu et al. demonstrated that green tea polyphenols promoted mRNA or protein expression of occludin after ischemia in the MCAO rat model by inhibiting PKC\textalpha\textsuperscript{ activity to reduce BBB leakage.\textsuperscript{[93]} The hairy root extract of Angelica gigas was proved to increase the expression of occludin in MCAO rats through activation of the PI3K/Akt pathway, leading to the alleviation of BBB disruption.\textsuperscript{[94]} Considering the positive outcomes, it is likely that multiple signaling pathways are involved in Chinese medicines mediated
occludin regulation in ischemic stroke, including the MAPK signaling pathway, PKCα activity, and PI3K/AKT signaling pathway. The effective and proven Chinese medicines could be considered as a therapeutic or preventative strategy for patients who have or are at high risk of suffering a stroke.

**Repurposing of conventional chemical drugs**

Recently, multitudes of evidence provide a new viewpoint for treating cerebral ischemic with conventional chemical drugs. For example, in addition to antispermatogenic function, adjudin had been shown that its anti-neuroinflammation effect plays an important role in preventing cerebral reperfusion injury in the tMCAO mouse model by suppressing the NF-κB pathway, inhibiting the elevated MMP-9 activity and increasing protein expression of occludin.[25] Moreover, the combination of NBO and minocycline (a broad-spectrum tetracycline antibiotics) significantly inhibited MMP-9 activity and occludin degradation in the rat MCAO model.[90] Furthermore, thalidomide, an old drug with anti-inflammatory and anti-cancer properties, downregulated the expression of TNF, IL-1 β, and MMP-9 that preserves occludin and attenuates BBB disruption.[93] These results show that certain conventional chemical drugs can be repurposed for preserving BBB integrity through inhibiting the NF-κB pathway, TNF, IL-1 β, or MMP-9 activity to increase occludin. However, further clinical studies are required to validate the effectiveness of these old drugs in treating stroke patients.

**Small molecular peptides**

Cystatin C, serves as a biomarker of renal function injury, widely exists in brain tissue, and the level of its expression increases in stroke. Yang et al. demonstrated that overexpression of cystatin C possesses neuroprotective effect on maintaining BBB integrity by increasing the expression of caveolin-1 and occludin after ischemic brain injury.[98] Although the treatment of cystatin C has rescued occludin by increasing caveolin-1 expression, the specific mechanism by which cystatin C regulates occludin will require additional investigation. N-acetylcysteine (NAC), a precursor of glutathione, contains free sulfhydryl of disulfide bonds and promotes synthesis of GSH. Due to its anti-oxidant and anti-inflammatory property, NAC is broadly applied to cardiovascular disease at present.[97] Wang et al. demonstrated that NAC in MCAO diabetic mice reduced occludin glycation and alleviated BBB permeability, showing cerebral protection by correcting the ratio of methylglyoxal/glutathione.[98] As an antioxidant, NAC offers a preventative approach to protect against the worsened stroke outcome in diabetics through reducing occludin glycation. Further clinical studies are still needed.

**Steroid hormones**

Vitamin D and glucocorticoid, as members of the steroid hormones family, bind to their receptors in the cytoplasm and nucleus, respectively, to trigger the strong biological effect. It was shown that exposure of neuronal cells to hypoxia/reoxygenation triggers a series of cascade reaction, including the increased formation of intracellular reactive oxygen species (ROS), increased activation of NF-κB signaling pathways, leading to augmented expression of MMP-9 that mediated BBB disruption through degradation of occludin, claudin-5, and ZO-1. Vitamin D treatment prevented BBB disruption by inhibiting ROS production and NF-κB activation in a vitamin D receptor-dependent manner.[99] However, another steroid hormone, the glucocorticoid stress hormone (GC), has the opposite effect. GC receptor signaling in endothelial cells can be activated under ischemic conditions, and GC reduced the expression level of occludin through binding to the GC receptor, contributing to worsening the ischemic infarct.[100] The role of steroid hormones acting on occludin has opposite views about regulating occludin expression through different signaling pathways, including inhibition of the NF-κB pathway or activation of the GC receptor. However, the basic study should further explore the specific mechanisms.

**Other therapies**

*In vitro* and *in vivo* evidence show that intravenous immunoglobulin could rescue ischemic neuronal cell by reducing leukocyte filtration and blocking BBB permeability, and importantly, it prevented the ischemia-induced downregulation of tight junction protein occludin and claudin-5.[101] This study demonstrated that immunoglobulin indeed can protect BBB by upregulating occludin, but the mechanisms are not clear. A recent study indicates that inhibition of miR-210 with its complementary locked nucleic acid oligonucleotides (miR-210-LNA)-mediated neuroprotection via preserving the expression of junction protein occludin in neonatal hypoxic-ischemic brain injury.[102] This result is limited to the role of miR-210 in a hypoxic-ischemic model. It is not clear whether miR-210-LNA treatment is effective in ischemic animal models, such as MCAO. Moreover, in an MCAO/R mice model, VEGF treatment aggravated BBB disruption by increasing LOC102640519 and HOXC13 through inhibition of ZO-1, occludin, and claudin-5,[103] which provides another therapeutic strategy for VEGF-based treatment for stroke patients.

Ischemic preconditioning (IP) has been shown to induce changes in tight junctions to protect against BBB breakdown after MCAO through activation ERK1/2.[104] In addition, the sphingosine kinase-2 contributes to protecting BBB integrity in hypoxic...
preconditioning-treated animals via generating S1P that participates in the maintenance of occludin at cytoskeletonally linked cell junctions.\textsuperscript{[108]} However, there have not enough clinical studies on IP alleviating BBB damage in ischemic stroke at present, and the role of IP in regulating occludin in clinic study needs to be done.

In addition, the emerging nanotechnology helps deliver beneficial drugs across the BBB. Some studies have reported that drugs loaded into the nanotechnology system could permeate through the BMECs.\textsuperscript{[106,107]} The mechanism for this delivery is most likely dependent on LDLs receptor-mediated endocytosis by the BMECs,\textsuperscript{[108]} and may not influence the tight junctions of the brain endothelium.\textsuperscript{[109]} Further studies on possible mechanisms will need to be explored in future.

**Conclusions**

In summary, occludin is a transmembrane protein of tight junctions that regulates the integrity and permeability of the BBB. Various factors have been found to regulate occludin expression, such as MMPs-dependent degradation, phosphorylation, ubiquitination, and other cytokines. The mechanisms of regulating occludin protein in tight junctions involve many diverse signaling pathways [Table 1], including NF-κB, MAPK, PKC, RhoK, and ERK1/2. Further, the different sites of phosphorylation or biological environments may impact

| Experimental model | Effect on occludin | Mechanism | Reference |
|--------------------|-------------------|-----------|-----------|
| Hypoxia mice model | Degradation       | Hypoxia-induced MMP-9 activation\textsuperscript{†} | [24] |
| tMCAO mice model  | Expression\textsuperscript{†} | MMP-9 activity \textsuperscript{†} by inhibition of NF-κB pathway | [25] |
| OGD/R-injury model | Degradation       | MMP-2/9 activity\textsuperscript{†} by activation of MAPK pathway | [26] |
| LLC-PK1 cells     | Thr-dephosphorylation | Activation of PKC | [32] |
| Caco-2 and MDCK cell monolayers | Thr-404, Thr-403 dephosphorylation Ser-490 Phosphorylation | Inhibition of PKC | [34] |
| BMECs             | Tyr-phosphorylation | Shear signal activation of Tiam1/Racl signal pathway | [39] |
| BMECs             | Tyr-phosphorylation and Thr-dephosphorylation | Activation of NMDA or AMPA/KA receptors | [41] |
| Microvascular endothelial cells | Tyr-dephosphorylation | Activation of PP2A | [42] |
| Sertoli cell      | Ubiquitination     | Dibutyryl-cAMP mediated the level of Itch and UBC4\textsuperscript{†} | [44] |
| Mpk-CCDc14 cells | Ubiquitination     | Nedd4-2 mediated immunoprecipitated | [45] |
| BRECcs            | Expression\textsuperscript{†} | VEGF-induced occludin ubiquitination | [46] |
| HCMEC/D3 cells    | TNF-α induced phosphorylation | Stimulation of p38MAPK and ERK1/2 | [49] |
| Renal endothelial cells | TNF-α induced expression\textsuperscript{†} | Activation of Rho/MLCK pathway | [50] |
| HCMEC/D3 cells    | TNF-α induced expression\textsuperscript{†} | Activation of Hif-1a/VEGF / VEGF-2/ERK | [51] |
| Human endothelial cells | TNF-α induced expression\textsuperscript{†} | PECM-1 redistribution | [52] |
| HBMECs and HAs    | TNF-α induced expression\textsuperscript{†} | NADPH oxidase activity\textsuperscript{†} | [53] |
| RPE cells         | IL-1β induced Expression\textsuperscript{†} | Unknown | [56] |
| HCMEC/D3 cells    | IL-1β induced expression\textsuperscript{†} | Activity of MMP-9 \textsuperscript{†} by activation of P2X7R | [57] |
| HCE cells         | IL-1β induced expression\textsuperscript{†} | Activity of NF-κB pathway | [58] |
| In vitro BBB model | IL-1β induced expression\textsuperscript{†} | Activation of NOTCH/NF-κB pathway | [60] |
| EAE mice model    | IFN-γ induced expression\textsuperscript{†} | Unknown | [65] |
| BALB/c mice model | IFN-γ induced expression\textsuperscript{†} | Unknown | [66] |
| HUVEC layers      | IFN-γ induced expression\textsuperscript{†} | Unknown | [67] |
| BBB transwell model | IFN-γ induced diassembled | Unknown | [68] |
| Cerebral embolism rats model | HGF-induced expression\textsuperscript{†} | Unknown | [73] |
| Human vascular endothelial cells | HGF-induced expression\textsuperscript{†} | Unknown | [74] |

MMP: Matrix metalloproteinase, IMCAO: Transient middle cerebral artery occlusion, NF-κB: Nuclear factor-κB, OGD/R: Oxygen-glucose deprivation/recovery, MAPK: Mitogen-activated protein kinase, PKC: Protein kinase C, LLC-PK1: The cell junctional complex in the pig kidney, Caco-2: Colorectal carcinoma, MDCK: Madin-Darby Canine Kidney, BREC: Bovine retinal endothelial cells, BMECs: Brain microvascular endothelial cells, NMDA: N-methyl-D-aspartate, AMPA/KA: Alpha-aminoo-3-hydroxy-5-methylisoxazole-4-propionate/kainate, PP2A: Protein phosphatase type 2A, mpk-CCDc14: Murine cortical collecting duct, HCMEC: Human cerebral microvascular endothelial cell, PECM-1: Platelet endothelial cell adhesion molecule-1, HBMECs: Human brain microvascular endothelial cells, NADPH: Nicotinamide adenine dinucleotide phosphate, ERK: Extracellular signal-regulated kinase, MLCK: Myosin light chain kinase, P2X7R: Ionotropic purinergic receptor, EAE: Experimental autoimmune encephalomyelitis, HUVEC: Human umbilical vein endothelial cell, TJs: Tight junctions, RPE: Racial pigment epithelial, HCE: Human corneal epithelial, BBB: Brain blood barrier, HGF: Hepatocyte growth factor, UBC4: Ubiquitin-conjugating enzyme 4, VEGF: Vascular endothelial growth factor, Hif-1: Hypoxia-inducible factor-1, Tiam1/Racl: Thymoma invasion and metastasis inducing factor1/ras-related C3 botulinumtoxin substrate, TNFα: Tumor necrosis factor-α, IL-1β: Interleukin-1β, IFN-γ: Interferon-γ.
the expression level of occludin. Occludin degradation has been considered as a subsequent event driven by stroke, including brain edema and hemorrhagic transformation. Therefore, occludin might be a potential biomarker for early hemorrhagic transformation in ischemic stroke. There are also numerous ischemic stroke treatments targeting BBB via occludin, which are summarized in Table 2. Moreover, clinical evidence suggests that NBO is a promising approach to expand the time window of reperfusion therapies in ischemic stroke. Although occludin has been shown to play a critical role in regulating BBB integrity, more preclinical studies are required to elucidate the roles of occludin before it can be considered a viable therapeutic target for ischemic stroke.

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Conflicts of interest
There are no conflicts of interest.

References
1. Petito CK, Feldmann E, Pulvinelli WA, Plum F. Delayed hippocampal damage in humans following cardiorespiratory arrest. Neurology 1987;37:1281-6.
2. Texel SJ, Zhang J, Camandola S, Unger EL, Taub DD, Koehler RC. Ceruloplasmin deficiency reduces levels of iron and BDNF in the cortex and striatum of young mice and increases their vulnerability to stroke. PLoS One 2011;6:e25077.
3. Liu R, Yuan H, Yuan F, Yang SH. Neuroprotection targeting ischemic penumbra and beyond for the treatment of ischemic stroke. Neurol Res 2012;34:331-7.
4. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev 2005;57:173-85.
5. Liu W, Hendren J, Qin XJ, Shen J, Liu KJ. Normobaric hyperoxia attenuates early blood-brain barrier disruption by inhibiting MMP-9-mediated occludin degradation in focal cerebral ischemia. J Neurochem 2009;108:811-20.
6. Yang Y, Estrada EY, Thompson JF, Liu W, Rosenberg GA. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rats. J Cereb Blood Flow Metab 2007;27:697-709.
7. Liu J, Jin X, Liu KJ, Liu W. Matrix metalloproteinase-2-mediated occludin degradation and caveolin-1-mediated claudin-5 redistribution contribute to blood-brain barrier damage in early ischemic stroke stage. J Neurosci 2012;32:3044-57.
8. Ren C, Li N, Wang B, Yang Y, Gao J, Li S, et al. Limb ischemic perconditioning attenuates blood-brain barrier disruption by inhibiting activity of MMP-9 and occludin degradation after focal cerebral ischemia. Aging Dis 2015;6:406-17.
9. Saunders NR, Dreifuss JJ, Dziegielewskia KM, Johansson PA, Habgood MD, Möllgard K, et al. The rights and wrongs of blood-brain barrier permeability studies: A walk through 100 years of history. Front Neurosci 2014;8:404.
10. Bentivoglio M, Kristensson K. Tryps and trips: Cell trafficking

Table 2: Occludin and ischemic stroke therapy

| Treatment or drugs | Experimental model | Mechanism | Occludin effect | Reference |
|-------------------|--------------------|-----------|-----------------|-----------|
| NBO plus tPA      | MCAO rats model    | Inhibition of MMP-9 activity | Expression† | [87]       |
| NBO               | MCAO rats model    | Inhibition of MMP-9 activity | Expression† | [5]        |
| NBO               | MCAO mice model    | Inhibition of gp91phox (or called Nox2) | Expression† | [89]       |
| NBO plus minocycline | MCAO rats model | Inhibition of on MMP-2/9 activity and apoptotic pathways | Expression† | [90]       |
| NBO               | Ischemic stroke patient | Inhibition of MMP-9 activity | Blood level↓ | [91]       |
| Cytopotashinone   | OGD/R injured NVU model | Down-regulation of MMP-9 activity | Expression† | [26]       |
| Gream tea polyphenols | MCAO rats model | Inhibition of PKCu activity | Expression† | [93]       |
| Angelica gigas    | MCAO rats model    | Activation of the PI3K/Akt pathway | Expression† | [94]       |
| Adjudin           | tMCAO mice model   | Suppression of the NF-κB | Expression† | [25]       |
| Minocycline       | MCAO rats model    | Inhibition of MMP-9 activity | Expression† | [90]       |
| Thalidomide       | MCAO mice model    | TNF-α, IL-1β production↑ and MMP-9 activity↑ | Expression† | [95]       |
| Cystatin C        | MCAO mice model    | Activity of MMP-9↓ | Expression† | [96]       |
| N-acetylcycteine  | Diabetic MCAO mice model | Anti-oxidant and anti-inflammatory | Occludin glycation↑ | [97]       |

NBO: Normobaric hyperoxia, MMP: Matrix metalloproteinase, tMCAO: Transient middle cerebral artery occlusion, OGD/R: Oxygen-glucose deprivation/recovery, NVU: Neurovascular unit, NF-κB: Nuclear factor κB, PKC: Protein kinase C, PI3K: Phosphatidylinositol 3-kinase, GC: Glucocorticoid, GR: Glucocorticoid receptor, ROS: Reactive oxygen species, HPC: Hypoxic preconditioning-induced cerebral, SP1: Sphingosine-1-phosphate, GSK3: Glycogen synthase kinase 3, MiR-210-3p: MicroRNA-210, LNA: Locked Nucleic Acid, VEGF: Vascular endothelial cell growth factor, TNF-α: Tumor necrosis factor-α, IL-1β: Interleukin-1β, Nox2: Niacin adenine dinucleotide phosphohydrolase

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across the 100-year-old blood-brain barrier. Trends Neurosci 2014;37:325-33.
11. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: An overview: Structure, regulation, and clinical implications. Neurobiol Dis 2004;16:1-3.
12. Greene C, Campbell M. Tight junction modulation of the blood brain barrier: CNS delivery of small molecules. Tissue Barriers 2016;4:e1138017.
13. Wollburg H, Lippoldt A. Tight junctions of the blood-brain barrier: Development, composition and regulation. Vascul Pharmacol 2002;38:323-37.
14. Zihni C, Mills C, Matter K, Balda MS. Tight junctions: From simple barriers to multifunctional molecular gates. Nat Rev Mol Cell Biol 2016;17:564-80.
15. Jiao H, Wang Z, Liu Y, Wang P, Xue Y. Specific role of tight junction proteins claudin-5, occludin, and ZO-1 of the blood-brain barrier in a focal cerebral ischemic insult. J Mol Neurosci 2011;44:130-9.
16. Correale J, Villa A. Cellular elements of the blood-brain barrier. Neurochem Res 2009;34:2067-77.
17. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, et al. Occludin: A novel integral membrane protein localizing at tight junctions. J Cell Biol 1993;123:1777-88.
18. Kuwabara H, Kokai Y, Kojima T, Takakuwa R, Mori M, Sawada N. Occludin regulates actin cytoskeleton in endothelial cells. Cell Struct Funct 2001;26:109-16.
19. Saitou M, Furuse M, Sasaki H, Schulze JD, Fromm M, Takano H, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol Biol Cell 2001;11:4131-42.
20. Bellmann C, Schreivogel S, Günther R, Dabrowski S, Schümann M, Wollburg H, et al. Highly conserved cysteines are involved in the oligomerization of occludin-redox dependency of the second extracelluar loop. Antioxid Redox Signal 2014;20:855-67.
21. Wittchen ES, Haskins J, Stevenson BR. Protein interactions at the tight junction. Actin has multiple binding partners, and ZO-1 forms independent complexes with ZO-2 and ZO-3. J Biol Chem 1999;274:35179-85.
22. Feldman GJ, Mullin JM, Ryan MP. Occludin: Structure, function and regulation. Adv Drug Deliv Rev 2005;57:883-917.
23. Hirase T, Staddon JM, Saitou M, Ando-Akatsuka Y, Itoh M, Toborek M. The NMDA and AMPA/KA receptors are involved in modulation of pTyr-occludin levels. J Cell Physiol 2002;192:1603-13.
24. Bauer AT, Bürgers HF, Rabie T, Marti HH. Matrix metalloproteinase-9 mediates hypoxia-induced vascular leakage in the brain via tight junction rearrangement. J Cereb Blood Flow Metab 2010;30:837-48.
25. Liu T, Zhang T, Yu H, Shen H, Xia W. Adjudin protects against cerebral ischemia reperfusion injury by inhibition of neuroinflammation and blood-brain barrier disruption. J Neuroinflammation 2014;11:107.
26. Zhao H, Zheng T, Yang X, Fan M, Zhu L, Liu S, et al. Cryptotanshinone Attenuates oxygen-glucose deprivation/recovery-induced injury in an in vitro model of neurovascular unit. Front Neurol 2019;10:381.
27. Yao X, Wang Y, Zhang D. microRNA-21 Confers neuroprotection against cerebral ischemia-reperfusion injury and alleviates blood-brain barrier disruption in rats via the MAPK signaling pathway. J Mol Neurosci 2018;65:43-53.
28. Chen YS, Chen KH, Liu CC, Lee CT, Yang CH, Chuang KC, et al. Propofol-induced vascular permeability change is related to the nitric oxide signaling pathway and occludin phosphorylation. J Biomed Sci 2007;14:629-36.
29. DeMaio L, Soukhazadeh M, Reddy S, Sevanian A, Hwang J, Hsiai TK. Oxidized phospholipids mediate occludin expression and phosphorylation in vascular endothelial cells. Am J Physiol Heart Circ Physiol 2006;290:H674-83.
30. Morgan L, Shah B, Rivers LE, Barden L, Groom AJ, Chung R, et al. Inflammation and dephosphorylation of the tight junction protein occludin in an experimental model of multiple sclerosis. Neuroscience 2007;147:664-73.
47. Kabra R, Knight KK, Zhou R, Snyder PM. Ned44-2 induces endocytosis and degradation of proteolytically cleaved epithelial Na+ channels. J Biol Chem 2008;283:6033-9.
48. McKenzie JA, Ridley AJ. Roles of Rho/ROCK and MLCK in TNF-alpha-induced changes in endothelial morphology and permeability. J Cell Physiol 2007;213:221-8.
49. Ni Y, Teng T, Li R, Simonyi A, Sun GY, Lee IC. TNFα alters occludin and cerebral endothelial permeability: Role of p38MAPK. PLoS One 2017;12:e0170346.
50. Xu C, Wu X, Hack BK, Bao L, Cunningham PN. TNF causes changes in glomerular endothelial permeability and morphology through a Rho and myosin light chain kinase-dependent mechanism. Physiol Rep 2015;3:e12636.
51. Zhang Y, Ding X, Miao C, Chen J. Propofol attenuated TNF-alpha-activated occludin expression by inhibiting Hif-1alpha/VEGF/VEGFR-2/ERK signaling pathway in hMEC/D3 cells. BMC Anesthesiol 2019;19:127.
52. Inyoo S, Suttithumrong A, Pattanakitsakul SN. Synergistic effect of TNF-α and dengue virus infection on adhesion molecule reorganization in human endothelial cells. Jpn J Infect Dis 2017;70:186-91.
53. Abdullah Z, Bayraktutan U. NADPH oxidase mediates TNF-α-evoked in vitro brain barrier dysfunction: Roles of apoptosis and time. Mol Cell Neurosci 2014;61:72-84.
54. Abdullah Z, Rakkar K, Bath PM, Bayraktutan U. Inhibition of TNF-α protects in vitro brain barrier from ischaemic damage. Mol Cell Neurosci 2015;69:65-79.
55. Allan SM, Tyrrell PJ, Rothwell NJ. Interleukin-1 and neuronal injury. Nat Rev Immunol 2005;5:629-40.
56. Abe T, Sugano E, Saigo Y, Tamai M. Interleukin-1beta and barrier function of retinal pigment epithelial cells (ARPE-19): Aberrant expression of junctional complex molecules. Invest Ophthalmol Vis Sci 2003;44:4097-104.
57. Yang F, Zhao K, Zhang X, Zhang J, Xu B. ATP induces disruption of tight junction proteins via IL-1 beta-dependent MMP-9 activation of human blood-brain barrier in vitro. Neural Plast 2016;2016:8928530.
58. Kimura K, Teranishi S, Nishida T. Interleukin-1beta-induced changes in glomerular endothelial permeability and morphology through a Rho and myosin light chain kinase-dependent mechanism. Physiol Rep 2015;3:e12636.
59. Fan LF, He PY, Peng YC, Du QH, Ma YJ, Jin JX, et al. Mdivi-1 ameliorates early brain injury after subarachnoid hemorrhage via the suppression of inflammation-related blood-brain barrier disruption and endoplasmic reticulum stress-based apoptosis. Free Radic Biol Med 2017;112:336-49.
60. Qin W, Li J, Zhu R, Gao S, Fan J, Xia M, et al. Melatonin protects blood-brain barrier integrity and permeability by inhibiting matrix metalloproteinase-9 via the NOTCH3/NF-kB pathway. Aging (Albany NY) 2019;11:1391-415.
61. Sun H, Tang Y, Guan X, Li L, Wang D. Effects of selective hypothermia on blood-brain barrier integrity and tight junction protein expression levels after intracerebral hemorrhage in rats. Biochim Biophys Acta 2013;1834:1317-24.
62. Zhou L, Sun X, Shi Y, Liu J, Luan G, Yang Y. Cysteinyl leukotriene receptor type 1 antagonist montelukast protects against injury of blood-brain barrier. Inflammopharmacology 2019;27:933-40.
63. Rozolimy VL, Markov AG. Effect of interleukin-1 beta on the expression of tight junction proteins in the culture of HaCaT keratinocytes. Bull Exp Biol Med 2010;149:280-3.
64. Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. Biochim Biophys Acta 2009;1788:864-71.
65. Ni C, Wang C, Zhang J, Qu L, Liu X, Lu Y, et al. Interferon-γ safeguards blood-brain barrier during experimental autoimmune encephalomyelitis. Am J Pathol 2014;184:3308-20.
66. Singh V, Kushwaha S, Gera R, Ansari JA, Mishra J, Dwangan J, et al. Sneaky entry of IFNγ through arsenic-induced leaky blood-brain barrier reduces CD200 expression by microglial pro-inflammatory cytokine. Mol Neurobiol 2019;56:1488-99.
67. Minagar A, Long A, Ma T, Jackson TH, Kelley RE, Ostanin DV, et al. Interferon (IFN)-beta 1a and IFN-beta 1b block IFN-gamma-induced disintegration of endothelial junction integrity and barrier. Endothelium 2003;10:299-307.
68. Rahman MT, Ghosh C, Hossain M, Linfield D, Rezaee F, Janigro D, et al. IFN-γ, IL-17A, or zonulin rapidly increase the permeability of the blood-brain and small intestinal epithelial barriers: Relevance for neuro-inflammatory diseases. Biochem Biophys Res Commun 2018;507:274-9.
69. Lopez-Ramirez MA, Fischer R, Torres-Badillo CC, Davies HA, Logan K, Pfitzenmaier K, et al. Role of caspases in cytokine-induced barrier breakdown in human brain endothelial cells. J Immunol 2012;189:3130-9.
70. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. J Biochem 1996;119:591-600.
71. Nakamura T, Mizuno S, Matsumoto K, Sawa Y, Matsuda H, Nakamura T. Myocardial protection from ischemia/reperfusion injury by endogenous and exogenous HGF. J Clin Invest 2000;106:1511-9.
72. Date I, Takagi N, Takagi K, Kago T, Matsumoto K, Nakamura T, et al. Hepatocyte growth factor attenuates cerebral ischemia-induced learning dysfunction. Biochem Biophys Res Commun 2004;319:1152-8.
73. Date I, Takagi N, Takagi K, Tanonaka K, Funakoshi H, Matsumoto K, et al. Hepatocyte growth factor attenuates cerebral ischemia-induced increase in permeability of the blood-brain barrier and decreases in expression of tight junctional proteins in cerebral vessels. Neurosci Lett 2006;407:141-5.
74. Jiang WG, Martin TA, Matsumoto K, Nakamura T, Mansel RE. Hepatocyte growth factor/scatter factor decreases the expression of occludin and transendothelial resistance (TER) and increases paracellular permeability in human vascular endothelial cells. J Cell Physiol 1999;181:319-29.
75. Nasu Y, Ido A, Tanoue S, Hashimoto S, Sasaki F, Kannura S, et al. Hepatocyte growth factor stimulates the migration of gastric epithelial cells by altering the subcellular localization of the tight junction protein ZO-1. J Gastroenterol 2013;48:193-202.
76. Mozaffarian D, Benjamin EL, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics-2016 update: A report from the American Heart Association. Circulation 2016;133:e38-60.
77. Khatri P, Wechsler LR, Broderick JP. Intracranial hemorrhage associated with revascularization therapies. Stroke 2007;38:431-40.
78. Kassner A, Roberts TP, Moran B, Silver FL, Mikulis DJ, Recombinant tissue plasminogen activator increases blood-brain barrier disruption in acute ischemic stroke: An MR imaging permeability study. AJNR Am J Neuroradiol 2009;30:1864-9.
79. Wang W, Li M, Chen Q, Wang J. Hemorrhagic transformation after tissue plasminogen activator reperfusion therapy for ischemic stroke: Mechanisms, models, and biomarkers. Mol Neurobiol 2015;52:1572-9.
80. Zhang C, An J, Haile WB, Echeverry R, Strickland DK, Yeps M. Microglial low-density lipoprotein receptor-related protein 1 mediates the effect of tissue-type plasminogen activator on matrix metalloproteinase-9 activity in the ischemic brain. J Cereb Blood Flow Metab 2009;29:1946-54.
81. Pan R, Yu K, Weatherwax T, Zheng H, Liu W, Liu KJ. Blood Occludin Level as a Potential Biomarker for Early Blood Brain Barrier Disruption Following Ischemic Stroke. Sci Rep 2017;7:40331.
82. Li W, Pan R, Qi Z, Liu KJ. Current progress in searching for clinically useful biomarkers of blood-brain barrier damage following cerebral ischemia. Brain Circ 2018;4:145-52.
83. Rosenberg GA. Neurological diseases in relation to the blood-brain barrier disruption in acute ischemic stroke: Mechanisms, models, and biomarkers. Mol Neurobiol 2015;52:1572-9.
model of transient focal cerebral ischemia. J Cereb Blood Flow Metab 2006;26:1274-84.
85. Liu W, Hendren J, Qin XJ, Liu KJ. Normobaric hyperoxia reduces the neurovascular complications associated with delayed tissue plasminogen activator treatment in a rat model of focal cerebral ischemia. Stroke 2009;40:2526-31.
86. Singhal AB. Oxygen therapy in stroke: Past, present, and future. Int J Stroke 2006;1:191-200.
87. Šalamon Š, Kramar B, Marolt TP, Poljšak B, Milisav I. Medical and Dietary Uses of N-Acetylcysteine. Antioxidants (Basel) 2019;8:111.
88. Henninger N, Fisher M. Normobaric hyperoxia-A promising approach to expand the time window for acute stroke treatment. Cerebrovasc Dis 2006;21:134-6.
89. Liu W, Chen Q, Liu KJ. Normobaric hyperoxia protects the blood brain barrier through inhibiting Nox2 containing NADPH oxidase in ischemic stroke. Med Gas Res 2011;1:22.
90. Jin X, Liu J, Liu KJ, Rosenberg GA, Yang Y, Liu W. Normobaric hyperoxia combined with minocycline provides greater neuroprotection than either alone in transient focal cerebral ischemia. Exp Neurol 2013;240:9-16.
91. Shi S, Qi Z, Pan R, Timmins GS, Zhao Y, et al. Normobaric Hyperoxia Reduces Blood Occludin Fragments in Rats and Patients With Acute Ischemic Stroke. Stroke 2017;48:2848-54.
92. Henninger N, Fisher M. Normobaric hyperoxia-A promising approach to expand the time window for acute stroke treatment. Cerebrovasc Dis 2006;21:134-6.
93. Liu X, Wang Z, Wang P, Yu B, Liu Y, Xue Y. Green tea polyphenols alleviate early BBB damage during experimental focal cerebral ischemia through regulating tight junctions and PKCalpha signaling. BMC Complement Altern Med 2013;13:137.
94. Oh TW, Park KH, Jung HW, Park YK. Neuroprotective effect of the hairy root extract of Angelica gigas NAKAI on transient focal cerebral ischemia in rats through the regulation of angiogenesis. BMC Complement Altern Med 2015;15:101.
95. Yoon JS, Lee JH, Tweedie D, Mughal MR, Chigurupati S, Greig NH, et al. 3,6'-dithiothalidomide improves experimental stroke outcome by suppressing neuroinflammation. J Neurosci Res 2013;91:671-80.
96. Yang B, Xu J, Chang L, Miao Z, Heang D, Pu Y, et al. Cystatin C improves blood-brain barrier integrity after ischemic brain injury in mice. J Neurochem 2020;153:413-25.
97. Šalamon Š, Kramar B, Marolt TP, Poljšak B, Milisav I. Medical and Dietary Uses of N-Acetylcysteine. Antioxidants (Basel) 2019;8:111.
98. Wang B, Aw TY, Stokes KY. The protection conferred against ischemia-reperfusion injury in the diabetic brain by N-acetylcysteine is associated with decreased dicarbonyl stress. Free Radic Biol Med 2016;96:89-98.
99. Won S, Sayeed I, Peterson BL, Wall B, Kahn JS, Stein DG. Vitamin D prevents hypoxia/reoxygenation-induced blood-brain barrier disruption via vitamin D receptor-mediated NF-kB signaling pathways. PLoS One 2015;10:e0122821.
100. Sorrells SF, Caso JR, Munhoz CD, Hu CK, Tran KV, Miguel ZD, et al. Glucocorticoid signaling in myeloid cells worsens acute CNS injury and inflammation. J Neurosci 2013;33:7877-89.
101. Widiapradja A, Santro T, Basta M, Sobey CG, Manzanero S, Arumugam TV. Intravenous immunoglobulin (IVIg) provides protection against endothelial cell dysfunction and death in ischemic stroke. Exp Transl Stroke Med 2014;6:7.
102. Ma Q, Dasgupta C, Li Y, Huang L, Zhang L. MicroRNA-210 suppresses junction proteins and disrupts blood-brain barrier integrity in neonatal rat hypoxic-ischemic brain injury. Int J Mol Sci 2017;18:1356.
103. Wu L, Ye Z, Pan Y, Li X, Xu F, Zhang B, et al. Vascular endothelial growth factor factor aggravates cerebral ischemia and reperfusion-induced blood-brain-barrier disruption through regulating LOC102640519/POX13/Z0-1 signaling. Exp Cell Res 2018;369:275-83.
104. Shin JA, Kim YA, Jeong SI, Lee KE, Kim HS, Park EM. Extracellular signal-regulated kinase 1/2-dependent changes in tight junctions after ischemic preconditioning contributes to tolerance induction after ischemic stroke. Brain Struct Funct 2015;220:13-26.
105. Wacker BK, Freie AB, Perfater JL, Gidday JM. Junctional protein regulation by sphingosine kinase 2 contributes to blood-brain barrier protection in hypoxic preconditioning-induced cerebral ischemic tolerance. J Cereb Blood Flow Metab 2012;32:1014-23.
106. Xie Y, Ye L, Zhang X, Cui W, Lou J, Nagai T, et al. Transport of nerve growth factor encapsulated into liposomes across the blood-brain barrier: In vitro and in vivo studies. J Control Release 2005;105:106-19.
107. Ishii T, Asat P, Oyama D, Fukuta T, Yasuda N, Shimizu K, et al. Amelioration of cerebral ischemia-reperfusion injury based on liposomal drug delivery system with asialo-erythropoietin. J Control Release 2012;160:81-7.
108. Kreuter J, Shamenkov D, Petrov V, Ramge P, Cychutek K, Koch-Brandt C, et al. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood-brain barrier. J Drug Target 2002;10:317-25.
109. Alyaudtin RN, Reichel A, Löbenberg R, Ramge P, Kreuter J, Begley DJ. Interaction of poly (butylcyanoacrylate) nanoparticles with the blood-brain barrier in vivo. J Drug Target 2001;9:209-21.