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REVIEW

Channels and Transporters in Ischemic Brain Edema

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Abstract: Brain edema is one of the most devastating consequences of acute ischemic stroke, but an effective and preventive therapy targeting the formation of ischemic brain edema has not yet to be validated. Ischemic brain edema is primarily caused by damage to the blood-brain-barrier, leading to the leakage of ions and water into the brain tissue. Ion channels and transporters play a crucial role in ischemic brain edema. Understanding their function and regulation mechanism could provide valuable insights into new therapeutic targets for ischemic brain edema. Recently, significant progress has been made in the study about ion channels and transporters as therapeutic targets for ischemic brain edema. In this review, we summarize ion channels and transporters involved in the formation of ischemic brain edema, including trigger force, physiological function, regulatory mechanisms, and detection technologies. Especially, we will focus on the roles of these ion channels and transporters in ischemic brain edema, as well as recent advances in therapeutic strategies targeting these transporters.

Keywords: acute ischemic stroke, brain edema, transporter, ion channel, blood-brain barrier

Introduction

Ischemic brain edema is a relative increase in the amount of water in the brain after acute ischemic stroke (AIS). An increase of water content leads to brain swelling in a fixed volume of skull and elevates intracranial pressure that may further cause brain herniation. Malignant brain edema (MBE) is defined as fast-progressing brain edema leading to rapid neurological deterioration, cerebral hernia, or death. MBE accounts for 80% mortality of large hemispheric ischemic strokes.[1] Even in patients with non-life-threatening stroke, the extent of brain edema is also a predictor of poor outcome.[2] Clinically, ischemic brain edema and resultant intracranial hypertension are treated with non-specific therapies such as hyperosmolar agents and, when severe, decompressive craniectomy. Although these therapies can be life-saving, they do not always improve functional outcomes, nor do they target or prevent the underlying pathobiological mechanisms. To date, the detailed mechanism of ischemic brain edema remains unclear. This limits advances in prevention and treatment strategies as well as drug development.

As a physical and biochemical barrier between the peripheral circulation and the central nervous system, the blood-brain barrier (BBB) restricts the free entry and exit of water, ions, plasma components into brain tissue. BBB contains transporters that provide nutrients to the central nervous system, ion channels that participate in brain ion homeostasis, and efflux transporters that prevent compounds from entering the brain.[3] Ion channels and transporters play a crucial role in ischemic brain edema. Understanding their function and regulation mechanism could provide valuable insights into new therapeutic targets for ischemic brain edema. The role of various transporters involved in the dysregulation of ionic homeostasis during ischemic brain edema was reviewed in 2009.[4] Over 10 years have passed, new advances have shed light on mechanical of ischemic brain edema, and have led to clinical trials of antagonists of key transporters in ischemic brain edema formation.[5]

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In this review, we summarize ion channels and transporters involved in the formation of ischemic brain edema, including trigger force, physiological function, regulatory mechanisms, and detection technologies. Especially, we will focus on the roles of these ion channels and transporters in ischemic brain edema, as well as recent advances in therapeutic strategies targeting these transporters.

Blood-Brain Barrier

BBB plays a vital role in maintaining the stability of the brain's internal environment. It is composed of continuous brain microvascular endothelial cells (BMECs), tight junctions between these cells, a complete basement membrane, pericytes, and a glial membrane surrounded by the end-feet of astrocytes (Figure 1). Among them, three types of cells play an important role in ensuring the structural and functional integrity of the BBB. There are numerous ion channels and transporters expressed on the membrane of the three cells to implement their functions.

BMECs have two major characteristics different from peripheral vascular endothelial cells. First, BMECs are connected to each other by tight junctions, which limits circulatory substances entering the brain by intercellular pathway. Second, BMECs have a negligible rate of vesicular transcytosis, which significantly reduce transportation by transcellular pathways.[6]

Due to contractile properties, pericytes regulate capillary diameter and brain blood flow. They can also remove harmful metabolites through phagocytosis. Importantly, pericytes express various vasoactive substances like

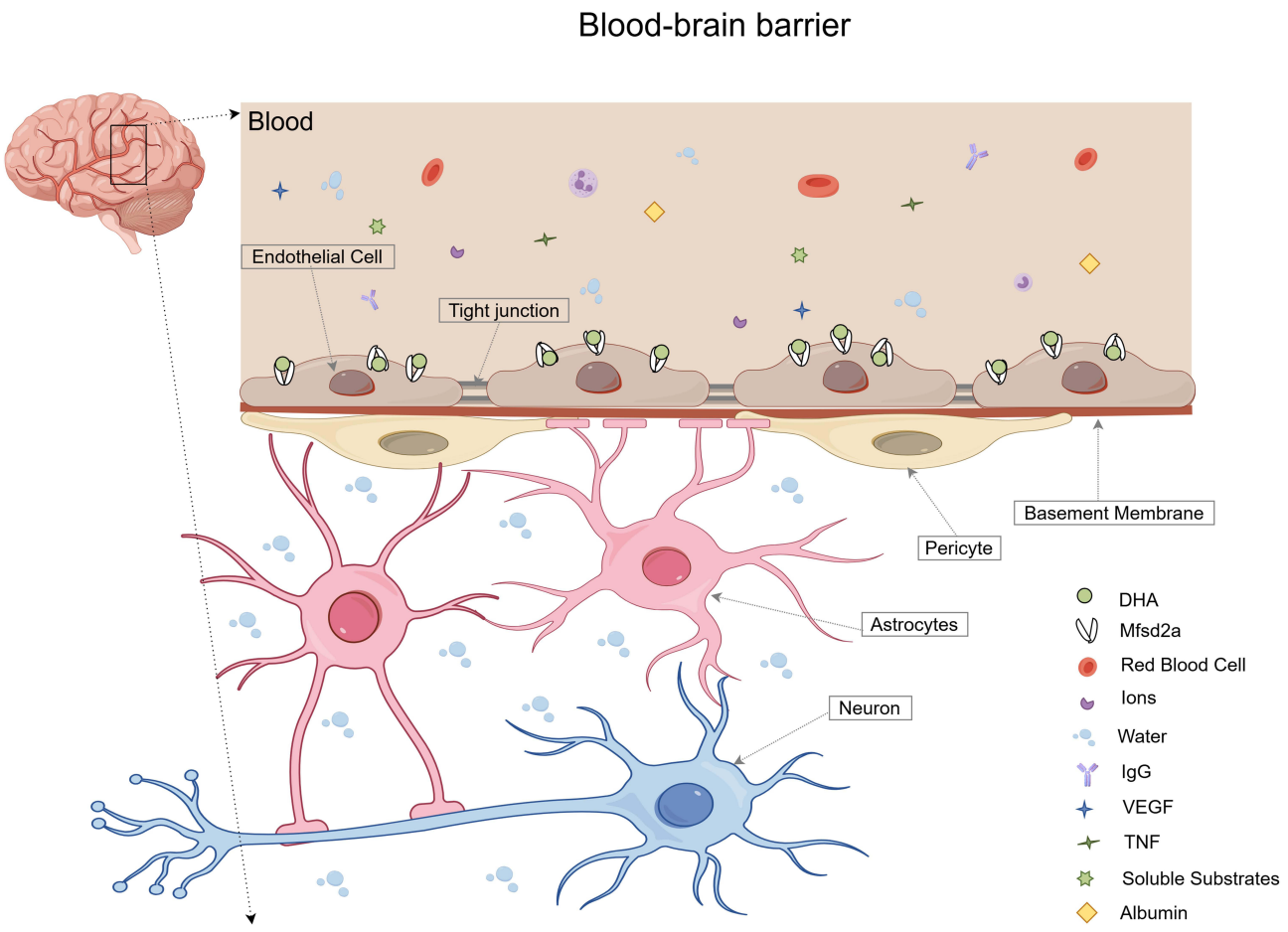

Figure 1 Blood-brain barrier. Normal blood-brain barrier is composed of continuous brain capillary endothelial cells, tight junctions between these cells, a complete basement membrane, pericytes, and a glial membrane surrounded by the end-feet of astrocytes. Tight junctions restrict blood components enter the brain through intercellular pathways. Mfsd2a reduces vesicular transport by transporting DHA, thereby limiting the entry of blood components into the brain through transcellular pathways.

Abbreviations: DHA, Docosahexaenoic Acid; Mfsd2a, major facilitator superfamily domain containing protein 2a; IgG, immunoglobin G; VEGF, Vascular endothelial growth factor; TNF, Tumor Necrosis Factor.

catecholamines, angiotensin I, vasoactive intestinal peptides, endothelin-1, and vasopressin.[7] These substances help control blood flow and vessel stability. Additionally, pericytes regulate the expression of specific genes in BMECs. For example, they control the expression of Mfsd2a, which is essential for maintaining BBB integrity.[8,9]

Astrocytes have unique end-feet, highly specialized and polarized structures with tentacle-like protrusions that cover BMECs and pericytes. These end-feet contain neatly arranged particles, including aquaporin-4 (AQP4) and the adenosine triphosphate (ATP)-sensitive inward rectifier potassium channel (Kir4.1).[10,11] These proteins help maintain water balance, ensure stable ion concentrations, and support hemoglobin function. Astrocytes also supply nutrients to neurons, regulate extracellular potassium levels, clean and circulate neurotransmitters, and manage immune system responses.

Ischemic Brain Edema

There are four fluid compartments within the brain: intravascular, interstitial, intracellular, and cerebrospinal fluid. Excessive water accumulation in any of these compartments can cause brain edema. Ischemic brain edema can be classified into three types base on the location water gathers: cytotoxic edema, ionic edema, and vasogenic edema.[12] Although three kinds of brain edema often occur simultaneously or continuously, they have unique molecular pathophysiology.

Cytotoxic edema is the first stage of ischemic brain edema, and emerges within several minutes after AIS. After cerebral ischemia and hypoxia, damage to the Na^+/K^+-ATPase disrupts the ion osmotic gradient between the inside and outside of the cell. As a result, water and ions, mainly Na^+, Cl^-, move from interstitial to intracellular space (Figure 2). This transfer is especially prominent in astrocytes, causing them to swell and setting the stage for ionic and vasogenic edema. Since both intracellular and interstitial spaces are part of brain tissue, this swelling does not increase brain volume, does not cause space-occupying effects, and is reversible. It appears as high signal in diffusion weighted imaging, and low signal in apparent dispersion coefficient imaging.[13] Channels and transporters, like Na^+-K^+-$2Cl^-$ cotransporter 1 (NKCC1),[14] AQP4,[15] Na^+/H^+ exchanger 1 (NHE1),[16] metabotropic glutamate receptor 5 (mGluR5),[7] Na^+-K^+-ATPase[17] play crucial role in cytotoxic edema (Figure 2).

Ionic edema occurs right after cytotoxic edema. The ion and charge gradient formed during cytotoxic edema causes Na^+, Cl^-, and water to move from the blood vessels into interstitial through BBB. This movement happens via intercellular and transcellular pathways. TJ decreases in the intercellular pathway, while vesicular transcytosis increases in the transcellular pathway (Figure 3). These changes involve complex signal transduction and ion channels, which are not yet fully understood. As ions and water enter interstitial space from intravascular space, the brain tissue volume increases causing significant swelling and damage to surrounding tissues. However, at this stage, the membranes of BMECs remain intact, preventing blood cells or large molecules from passing through. Channels and transporters, like major facilitator superfamily domain-containing protein 2 (Mfsd2a),[8] transient receptor potential melastatin 4 (TRPM4),[18] volume-regulated anion channel (VRAC),[19] Connexin 43 (Cx43),[20] NKCC1, Kir4.1,[21] play crucial role in ionic edema (Figure 3).

Vasogenic edema is caused by damage to the brain's blood vessels (BMECs), leading to the breakdown of the BBB. This breakdown allows large molecules to leak from blood vessels to brain tissue (Figure 4). Vasogenic edema occurs several hours after AIS. As edema progress, blood cells can also enter the brain tissue, causing hemorrhagic transformation, which is the most severe form of cerebral edema.

In the next section, we will elaborate the major ion channels and transporters one by one, focus on their roles in ischemic brain edema, as well as recent advances in therapeutic strategies targeting these transporters.

AQP4

Aquaporin are membrane proteins that allow bidirectional movement of water molecules across the phospholipid bilayer plasma membrane. There are 14 different members, with AQP4 being the most abundant aquaporin in center nervous system (CNS).[22] AQP4 is primarily found in astrocytes and the ependyma within CNS. It is predominantly expressed at brain-fluid interfaces, including the end-feet of astrocytes.[23] This strategic localization allows AQP4 to efficiently regulate water movement within the brain.

AQP4 plays a critical and complex role in ischemic brain edema. After AIS, the expression of AQP4 is significantly upregulated.[23] This upregulation is a response to the disruption of the BBB and the accumulation of fluid in the brain parenchyma. In the early stages of AIS, the increased AQP4 expression promotes water influx into the brain tissue,

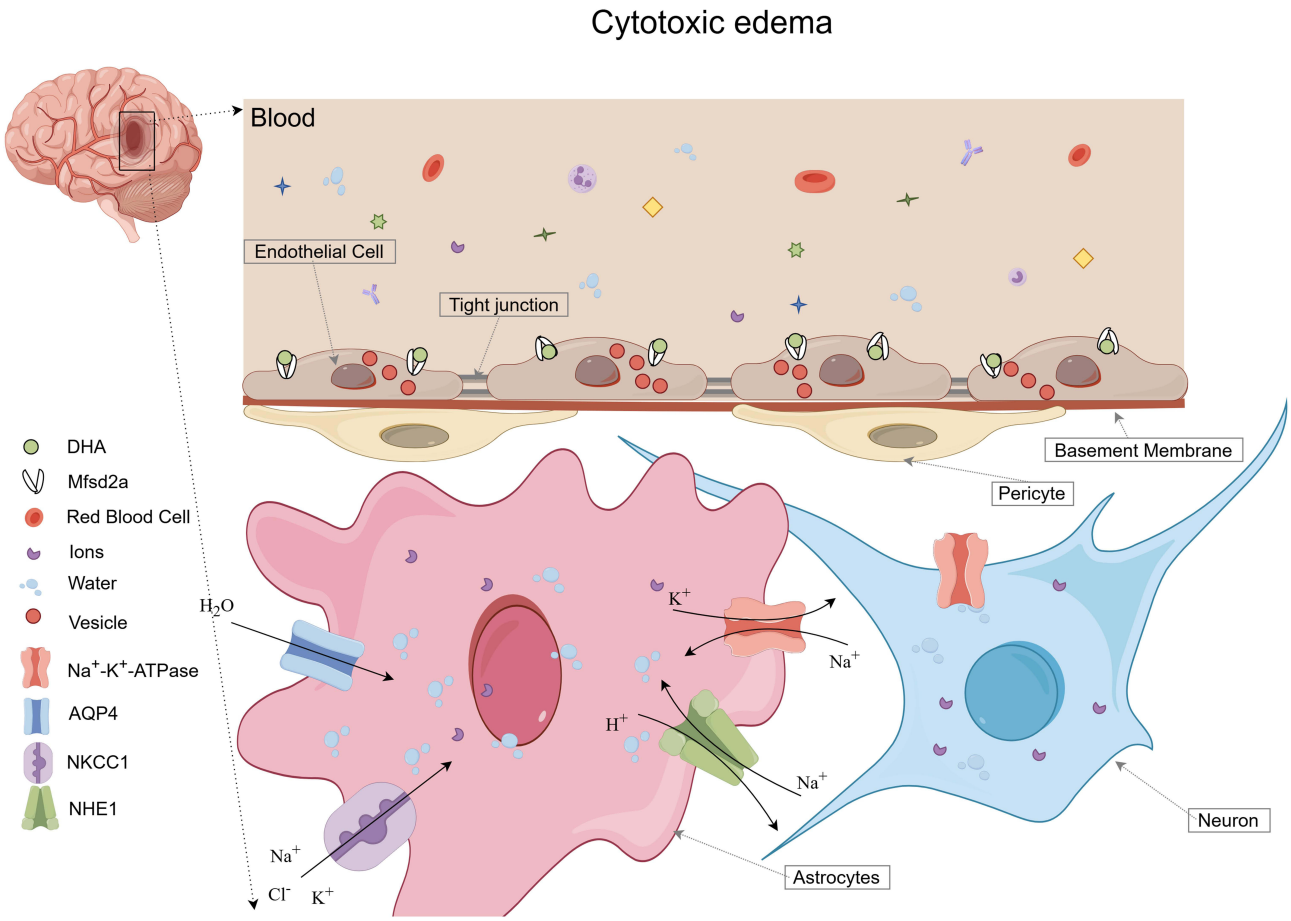

Figure 2 Cytotoxic edema. Transporters like AQP4, NHE1, NKCC1, Na^+-K^+-ATPase, and mGluR5 are active by ischemia several minutes after onset. Water and ions enter neurons and glial cells from the intercellular space, causing swelling and deformation of the cells, but without significant space occupying effects.
Abbreviations: DHA, Docosahexaenoic Acid; Mfsd2a, major facilitator superfamily domain containing protein 2a; AQP4, aquaporin Protein-4; NHE1, Recombinant Sodium/Hydrogen Exchanger 1; NKCC1, Na^+-K^+-2Cl- cotransporter 1; Na^+-K^+-ATPase, Sodium-Potassium Pump.

contributing to the formation of cytotoxic edema.[24,25] In the later stages, AQP4 aids in the clearance of excess water, thereby reducing vasogenic edema. This dual role suggests that precise timing and regulation of AQP4 activity are crucial for effective therapeutic interventions.[24,25] AQP4 interacts with several proteins and channels crucial for ischemic brain edema. The roles of these channels and their interactions with AQP4 will be discussed in subsequent sections.

Experimental studies using AQP4 knockout mice or AQP4 inhibitors have shown a reduction in ischemic brain edema formation.[26,27] Research has focused on developing AQP4 inhibitors as potential therapeutic agents for reducing ischemic brain edema.[27] Although only preliminary data from conference abstracts have reported the use of AQP4 inhibitors like AER-270 and AER-271 in experimental models,[28,29] these findings highlight the therapeutic potential of targeting AQP4 to mitigate ischemic brain edema. Acetazolamide and antiepileptic drugs have been used to target AQP4, but have not been demonstrated to be clinically useful for the treatment of brain swelling. 2-(nicotinamide)-1,3,4-thiadiazole (TGN-020), a specific AQP4 inhibitor, was shown to have beneficial effects in ischemic brain edema in rodents.[30] Calmodulin is an essential component of AQP4 translocation to the membrane. Trifluoperazine, a calmodulin antagonist, had beneficial effects on spinal cord injury edema and enhanced functional recovery in rats.[31]

TRPV4

Transient Receptor Potential Vanilloid 4 (TRPV4) is a calcium-permeable ion channel that responds to changes in osmotic pressure, temperature, and mechanical stress. TRPV4 can be activated by cell swelling, leading to an influx of calcium ions during cerebral ischemia. This influx can exacerbate cellular injury and inflammation, worsening ischemic brain edema.[15] TRPV4 has been found to form a complex with AQP4 through physical interaction, playing a complex

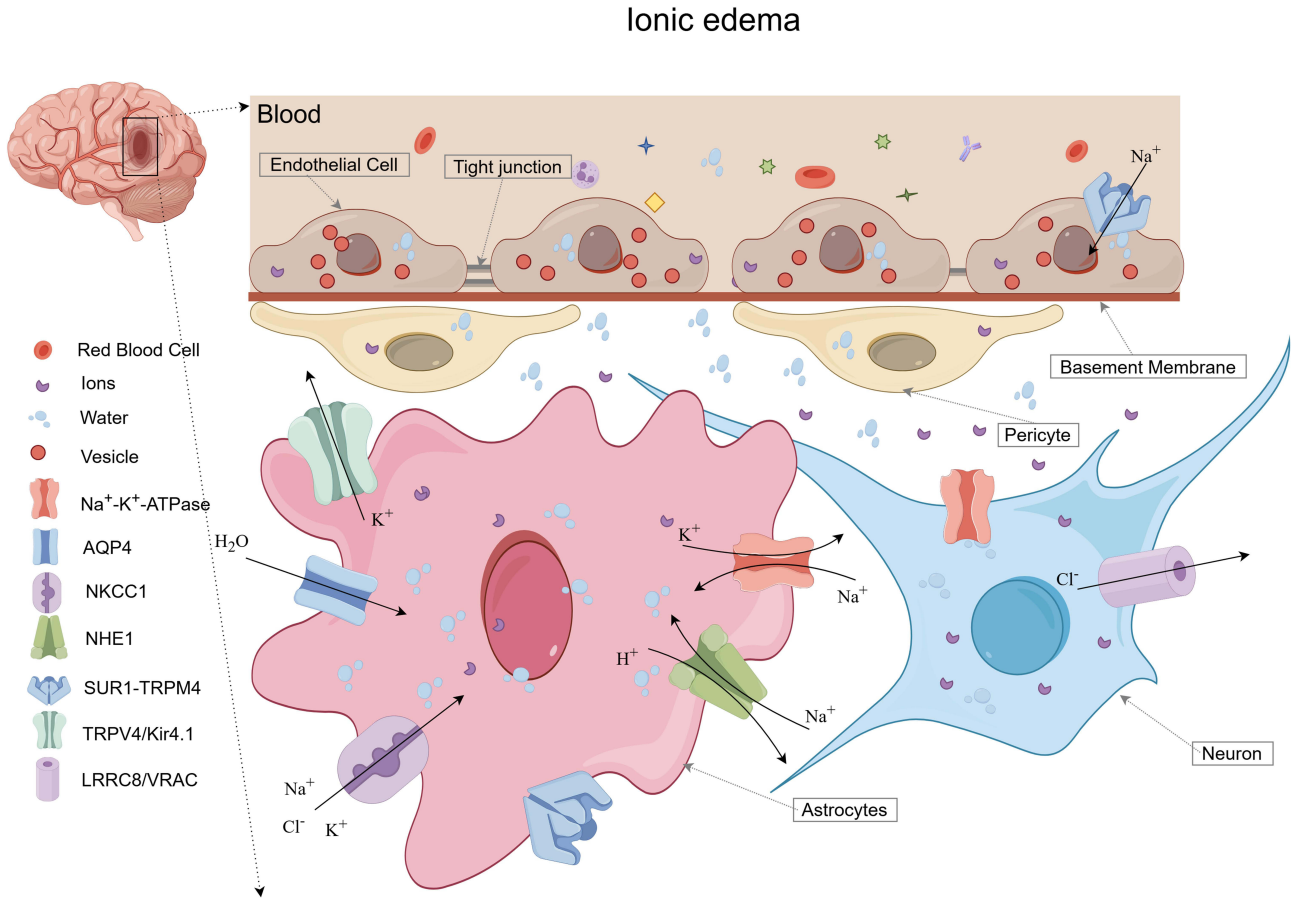

Figure 3 Ionic edema. Mfsd2a significantly reduced, vesicular transcytosis increase, and tight junction decrease. Water and ions move from the blood vessels into interstitial by intercellular and transcellular pathway, with significant occupancy effect. Kir4.1, NKCC1, SUR1/TRPM4, Mfsd2a, LRRC8/VRAC play an important role.
Abbreviations: DHA, Docosahexaenoic Acid; Mfsd2a, major facilitator superfamily domain containing protein 2a; AQP4, aquaporin Protein-4; NHE1, Recombinant Sodium/Hydrogen Exchanger 1; NKCC1, Na^+-K^+-2Cl- cotransporter 1; Na^+-K^+-ATPase, Sodium-Potassium Pump; Kir4.1, Inwardly rectifying potassium channel subtype 4.1; SUR1/TRPM4, Sulfonylurea Receptor 1/Transient Receptor Potential Melastatin 4; LRRC8/VRAC, Leucine Rich Repeat Containing Protein 8A/Volume-regulated anion channel.

role under both normal physiological and pathological conditions.[32] The relationship between AQP4 and TRPV4 has significant implications for their overall functions in various brain regions.

TRPV4 knock out mice exhibited reduced lesion volume and decreased Evans blue leakage following ischemic injury.[33] However, inhibiting TRPV4 with either gadolinium or ruthenium red in cultured astrocytes decreased Ca^{2+} influx but did not affect cell volume.[34] Specific TRPV4 activation induced dose-dependent apoptosis, neuronal death, and glial activation in cell cultures and a middle cerebral artery occlusion model.[35] Furthermore, TRPV4 activation with 4α-phorbol 12,13-didecanoate (4α PDD) reduced infarct volume and improved functional outcomes in the middle cerebral artery occlusion stroke model, resulting in a 3.4-fold increase in microvessel density and enhanced neurogenesis.[36]

These conflicting findings regarding whether TRPV4 inhibition or activation is more beneficial in ischemic stroke may stem from differences in experimental parameters across studies. The role of TRPV4 may also vary among cell types, such as proliferation in neurons and endothelial cells, and inflammation in microglia and astrocytes. Additionally, variations could arise from the nature and severity of the disease. This underscores the need for a comprehensive understanding of diseases mechanisms and the specific cell types involved before developing treatment options.

TRPM4

TRPM4 is a monovalent cation permeable channel, which is de novo synthesized after center nervous system injury and activated by ATP depletion.[37] CNS injury triggers the activation of the hypoxia-inducible factor 1 transcription factor, which promotes the transcription and expression of TRPM4 in neurons, glial cells and BMECs.[37] When expressed, TRPM4 is believed to form

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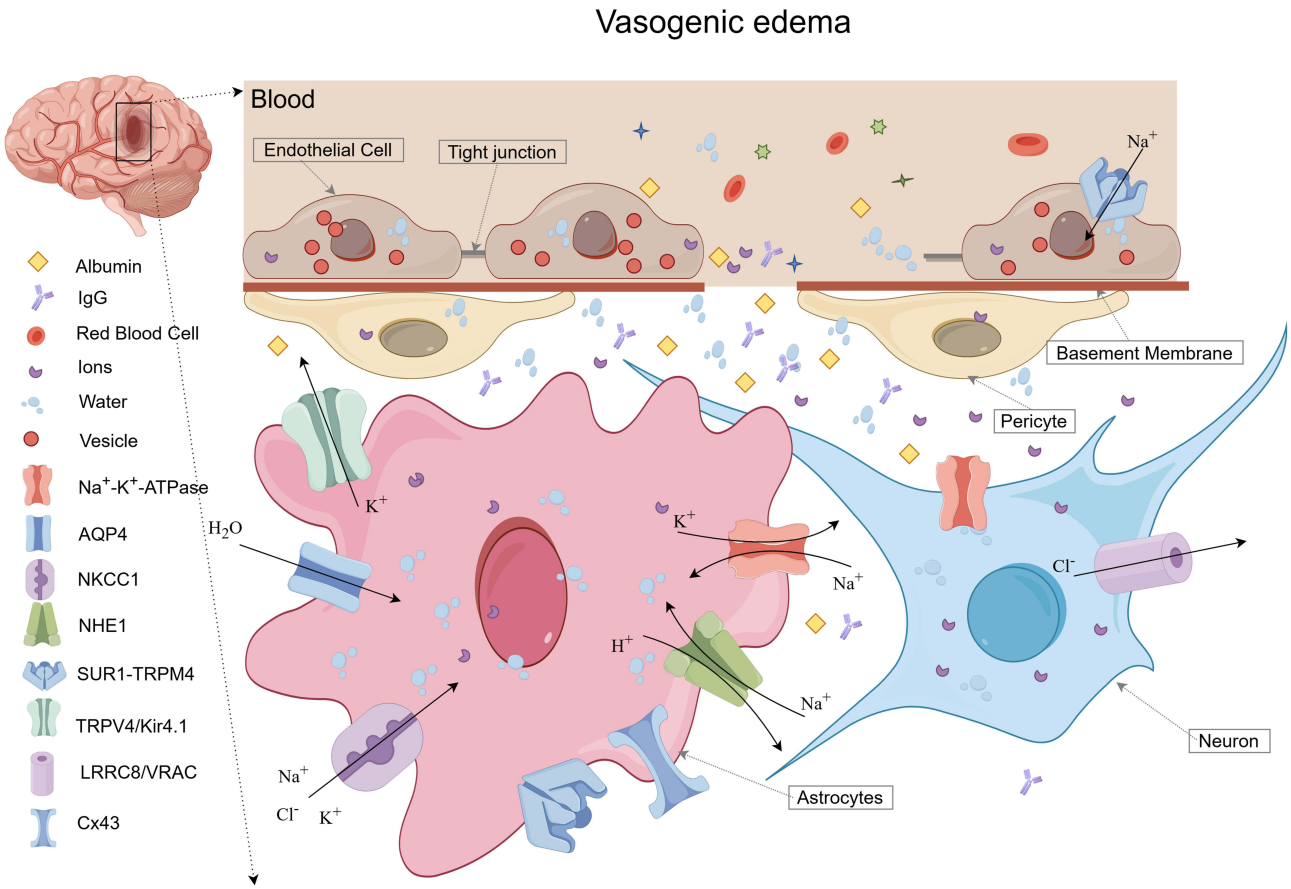

Figure 4 Vasogenic edema. Brain's blood vessels are damaged, large molecules leak from blood vessels to brain tissue.
Abbreviations: DHA, Docosahexaenoic Acid; NHE1, Recombinant Sodium/Hydrogen Exchanger 1; NKCC1, Na^+-K^+-2Cl- cotransporter 1; Na^+-K^+-ATPase, Sodium-Potassium Pump; Kir4.1, Inwardly rectifying potassium channel subtype 4.1; SUR1/TRPM4, Sulfonylurea Receptor 1/Transient Receptor Potential Melastatin 4; LRRC8/VRAC, Leucine Rich Repeat Containing Protein 8A/Volume-regulated anion channel; Cx43, connexin 43.

complexes with sulfonylurea receptor 1 (SUR1) and this complex has been implicated in ischemic brain edema.[38] Blocking SUR1 with low-dose glibenclamide reduced ischemic brain edema, infarct volume and mortality by 50% in rodent models of AIS.[39] However, retrospective clinical trials in AIS patients treated with sulfonylureas have yielded inconsistent results.[40,41] Stokum et al elucidated a mechanism involving the formation of a heteromultimeric complex of TRPM4-SUR1-AQP4, which acts as a key ion and water transporter complex responsible for astrocyte swelling.[22]

Different from other transport proteins, the de novo synthesis of TRPM4 makes it a highly promising target for treating brain edema. Professor Marc Simard conducted a series of prospective intervention trials on the anti-hematoma effect of glibenclamide.[42–48] Several options are being developed to target TRPM4 including specific chemical inhibitors,[49] and TRPM4 blocking antibodies.[50]

NKCC1

NKCC1 is a membrane protein that belongs to the cation-chloride cotransporter family. It is widely expressed in various cell types within the CNS, including neurons, astrocytes, and BMECs. NKCC1 actively transports Na^+, K^+, and Cl^- ions into cells, which is essential for regulating cell volume and the maintaining osmotic balance.[17]

During AIS, blood flow disruption reduces oxygen and glucose supply, causing energy failure and membrane depolarization. This impairs ion pumps, leading to an accumulation of intracellular Na^+ and Ca^{2+} ions. NKCC1 activity further contributes to this ion imbalance by transporting Na^+ and Cl^- into cells. This ion buildup creates an osmotic gradient that draws water into cells, causing swelling and cytotoxic edema. NKCC1's role in ischemic brain edema is complex and varies by cell type. It mainly contributes to cytotoxic edema in astrocytes and neurons,[17] while in BMECs, it may also affect vasogenic edema by influencing ion transport and barrier integrity.[51]

Bumetanide, a small molecule with excellent delivery across the BBB, is a relatively specific inhibitor of NKCC1 at low concentrations. Experimental studies using NKCC1 knockout mice or specific NKCC1 inhibitors (eg, bumetanide) have shown a reduction in ischemic brain edema.[52] Several clinical trials have examined the efficacy of bumetanide for the treatment of neurological disorders, including neonatal seizures,[53,54] Parkinson disease,[55,56] Alzheimer's disease,[57] autism spectrum disorder,[58–61] seizures.[53] However, there are no clinical trials available evaluating its effect on ischemic brain edema.

Na^+-K^+-ATPase

The Na^+-K^+-ATPase, also known as Na pump, or simply "the pump", is part of the P-type ATPase family. These proteins use energy from the ATP hydrolysis and become temporarily phosphorylated to move ions across cell membranes.[62] Na^+-K^+-ATPase is a large, highly expressed membrane protein complex (most cells contain over one million surface pumps per cell). It is an enzyme that actively transports Na^+ out of the cell in exchange for K^+, maintaining their concentration gradients across the cell membrane.[63]

Na^+-K^+-ATPase is crucial for maintaining of intracellular electrolyte homeostasis. During AIS, reduced blood flow leads to a lack of oxygen and glucose, causing cellular energy failure. This energy failure impairs Na^+-K^+-ATPase function, leading to the accumulation of intracellular Na^+ and a reduction in K^+ efflux. This ion imbalance creates an osmotic gradient that drives water into the cells, causing cellular swelling and cytotoxic edema.[64] Na^+-K^+-ATPase helps maintain the integrity of the BBB by regulating ion and water movement across BMECs. Disruption of its activity can lead to increased BBB permeability. This change allows plasma components to enter the brain parenchyma, leading to vasogenic edema.[17]

The Na^+-K^+-ATPase plays an important role in ischemic brain edema. Treatments that enhance Na^+-K^+-ATPase activity or mitigate their inhibition may help to reduce ischemic brain edema. Potassium aspartate (PA) is an electrolyte supplement, which has a strong affinity with cells. PA improves ischemic brain edema by increasing Na^+-K^+-ATPase activity.[65]

Kir4.1

Kir4.1 is a potassium channel primarily expressed in astrocytes, essential for maintaining potassium ion homeostasis and regulating extracellular potassium levels. In AIS, Kir4.1 helps prevent cellular swelling by facilitating potassium ion efflux, maintaining osmotic balance, and protecting against astrocyte swelling.[15] Both Kir channels and Na^+/K^+ ATPase are crucial for preserving normal extracellular composition, although they operate within different time frames.[66] Kir4.1 has been shown to interact with AQP4,[15] the significance of this interaction for cell swelling remains debated.[67] Some studies indicated that AQP4-null astrocytes exhibit increased connectivity through gap junctions and enhanced K^+ spatial buffering.[68] The functional interactions and regulation of these transporters under normal and pathological conditions require future investigation.

Mfsd2a

Mfsd2a is a transporter protein that belongs to the major facilitator superfamily. It has 12 transmembrane domains, which is typical for the major facilitator superfamily transporters. Mfsd2a is predominantly expressed in BMECs.[69] It has two main functions, fatty acid transport and maintaining low transcytosis. First, Mfsd2a transport omega-3 and omega-6 fatty acids, which are crucial for brain development and function.[70] In Mfsd2a knockout mice, the brain's content of docosahexaenoic acid is significantly reduced and associated with neuron loss, resulting in microcephaly and cognitive impairment.[70] Based on the role of Mfsd2a in the CNS, it has been preliminarily suggested as a potential therapeutic target for drug delivery to the CNS.[71] Second, Mfsd2a is the only known molecule that inhibits caveolae-mediated endocytosis, which is vital for maintaining BBB.[8,72] Mfsd2a gene knockout leads to an increase in the permeability of the BBB from embryonic stages to adulthood while maintaining the normal pattern of the vascular network.[73]

After CNS injuries such as ischemia, Mfsd2a expression can be affected. Studies have shown that Mfsd2a is involved in various acute and chronic CNS diseases, such as microcephaly,[74] Alzheimer's disease,[75] chronic alcohol abuse,[76] intracranial hemorrhage,[77] subarachnoid hemorrhage,[78] chronic cerebral ischemia,[79] surgical brain injury.[80] A reduction in Mfsd2a function or expression can exacerbate BBB breakdown and contribute to the development of brain edema.[80] Conversely, enhancing Mfsd2a function could be a potential therapeutic strategy to strengthen the BBB and reduce the risk of brain edema following CNS injuries. However, there is currently no drug research targeting Mfsd2a specifically

for treating ischemic brain edema. Research is ongoing to fully understand the regulatory mechanisms of Mfsd2a and its potential as a therapeutic target for conditions leading to ischemic brain edema.

Other Channels and Transporters and Their Interactions

High levels of extracellular glutamate release after AIS can cause astrocyte swelling and brain edema,[81] primarily through the activation of mGluR5,[7] a postsynaptic receptor widely expressed in the central nervous system. This activation leads to various cellular responses, including altered ion channel activity and intracellular signaling pathways that contribute to ischemic brain edema. Na^+-dependent excitatory amino acid transporters (EAATs) in presynaptic neurons and glia help maintain lower extracellular glutamate levels. Na^+-$HCO3^-$ cotransporters (NBCs) support astrocyte survival during AIS by alleviating intracellular acidosis. However, increased NBC activity can also affect membrane potential and promote excitability.[82] Glucose Transporters (GLUTs) and Sodium-Glucose Linked Transporter 1(SGLT1) are crucial for supplying energy to neurons and astrocytes, and disruptions in glucose transport after AIS can lead to energy deficits and exacerbate cell swelling.[83,84] Connexin proteins, particularly Cx43, form gap junctions and open hemichannels, facilitating the release of inflammatory mediators.[20] The known transporters associated with ischemic brain edema are shown in Table 1.

Interactions between channels and transporters lead to the formation of heteromultimeric structures with specific functions. The AQP4/TRPV4 complex facilitates regulatory volume decrease to counteract astrocyte swelling.[32] In contrast, the AQP4/TRPM4 complex promotes water influx and cell swelling in response to injury.[22] In a series of studies, it was shown that when TRPM4 is expressed, AQP4 shifts its association from TRPV4 to TRPM4, coinciding with the movement of the AQP4 from astrocyte end-feet to the plasma membrane of the cell body. Additionally, Na^+/K^+ ATPase interacts with AQP4 and mGluR5, contributing to swelling after injury.[15] Although NKCC1 is known to functionally interact with AQP4, it primarily mediates regulatory volume increase through influx of water.[96]

Discussion

Channels and transporters play a crucial role in the process of ischemic brain edema. Understanding the role of these membrane proteins can provide insights into the mechanisms of fluid accumulation in the ischemic brain and may open avenues for potential therapeutic interventions. Current studies explore various aspects of channels and transporters in ischemic brain edema, some of which may present conflicting findings, and most of which cannot be translated into clinical trials. During the review process, we identified several noteworthy issues in future research.

First, the methods for evaluating brain edema vary significantly across studies. As the most common method for evaluating brain edema in animal experiments, dry-wet weight lacks sufficient accuracy. Additionally, the procedures for drying, calculating, and expressing results are inconsistent. Some studies utilize different staining agents to visualize the volume or area of ischemic brain tissue. However, these methods unable to differentiate between infarction and edema. A few studies assess the degree of brain edema using magnetic resonance imaging in animal. However, this technique is rarely employed due to limited availability and the lack of standardized parameters. Inconsistent evaluation methods may be the primary cause of conflicting research findings. Therefore, future studies should focus on improving and standardizing the parameters for evaluating cerebral edema.

Second, a comprehensive understanding of diseases mechanisms and the specific cell types or brain region involved is needed before developing treatment options. Third, the formation process of ischemic cerebral edema involves multiple mechanisms and transporters, therefore, therapy of multi-target or heteromultimeric may be more effective. Forth, designing experiments based on the stroke preclinical assessment network (SPAN)[97] is more likely to lead to clinical translation, including multicenter, randomized, double-blind, and other approaches.

Conclusion

The development of ischemic cerebral edema is a series of continuous and complex processes. Channels and transporters play a crucial role in this process. Understanding the role of these membrane proteins can provide insights into the mechanisms of fluid accumulation in the ischemic brain and may open avenues for potential therapeutic interventions. This article helps to clarify the role of common membrane proteins in ischemic brain edema.

Table 1 Characteristics of Ion Channels and Transporters in Ischemic Brain Edema

Ion and Transporter	Cell Types	Trigger	Transporting Objects	Passive/ Active	Change During Ischemia	Agonists	Inhibitor
AQP4	Astrocytes, ependyma		Water	Passive	Up regulated		TGN-020,[85] IMD-0354[86]
TRPV4	All types of cells in the brain	Hypoosmotically induced cellular swelling	Calcium	Passive	Up regulated	GSK1016790A, 4αPDD, RN-1747[34]	PIP2, Gd3+, La3+, Capsazepine, ruthenium red, HC-067047, RN-1734[34]
SUR1/ TRPM4	All types of cells in the brain	Nanomolar concentrations of intracellular Ca2+ and intracellular ATP (EC50, ~1 μM)	Monovalent cations (Na^+)	Passive	De novo upregulation		Glibenclamide,[87] CBA[49]
NKCC1	Glia, neurons, BMECs, and epithelial cells of the choroid plexus	Elevated extracellular potassium and glutamate levels	Cation-chloride	Active	Up regulated		Ethacrynic Acid,[88] Furosemide, bumetanide[15]
Na^+-K^+-ATPase	Astrocytes		Sodium	Active	Down regulated	PA[65]	Ethacrynic acid[88]
Kir4.1	Glial	A decrease in cellular ATP levels or an increase in ADP levels	Potassium	Active	Down regulated	Luteolin[89]	
Mfsd2a	BMECs		DHA	Active	Down regulated		Tunicamycin[69]
mGluR5	Glutamatergic pyramidal neurons, GABAergic neurons, glial		Glutamate	Active	Up regulated	VU0360172[90]	mGlu5 NAM (MTEP, fenobam and AFQ056)[90]
EAAT1	Glia, BMECs, choroidal cells	A sudden Glutamate excess in the CSF	Glutamate	Active	Down regulated		TFBTBOA[91]
EAAT2	Glia, neurons, BMECs, choroidal cells	A sudden glutamate excess in the CSF	Glutamate	Active	Down regulated		TFBTBOA[91]
NBC	Glia	Neuronal activity and the subsequent K+ influx	Sodium-bicarbonate	Active	Up regulated		S0859[92]
GLUT1	Glia, BMECs	The D-glucose concentration gradient between blood and brain interstitium	D-glucose	Passive	Up regulated	Estrogen, Ascorbic acid, Curcumin[83]	
GLUT2	Neurons, glia oligodendrocyte, ependymal cells and tanycytes	The D-glucose concentration gradient between blood and brain interstitium	D-glucose	Passive	Up regulated		
SGLT1	In neurons throughout the brain	Hypoglycemic and hypoxemic	Sodium-D-glucose	Active	Up regulated		Sotagliflozin[93]

(*Continued*)

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Table 1 (Continued).

Ion and Transporter	Cell Types	Trigger	Transporting Objects	Passive/ Active	Change During Ischemia	Agonists	Inhibitor
Cx43	Glia, BMECs	The presence pro-inflammatory cytokines, increase in intracellular calcium concentration, metabolic inhibition	Critical ion, ATP and gliotransmitters	Active	Controversial		Leptin, CBX, Cx43 mimetic peptide (Gap 19, Gap 26, Gap 27, peptide 5, and L2 peptide)[20]
LRRC8/ VRAC	Glial	Hypotonic challenge or swelling	Chloride, organic osmolytes	Active	Up regulated		DCPIB[19]
NHE1	All types of cells in the brain	Hypoxemic	Sodium	Passive	Up regulated		HOE642, Rimeporide[94]
CIC-2	Astrocytes, oligodendrocytes, pyramidal hippocampal neurons and interneurons	Membrane hyperpolarization, hypotonic-induced cell swelling	Chloride	Passive	Up regulated		DIDS[95]

Abbreviations: AFQ056, 1H-indole-1-carboxylic acid, octahydro-4-hydroxy-4-(2-(3-methylphenyl)ethynyl; ATP, adenosine triphosphate; AQP4, aquaporin-4; BMECs, brain capillary endothelial cells; CBA, 4-chloro-2-(2-chlorophenoxy) acetamido benzoic acid; CBX, carbenoxolone; CIC-2, chlorine channel2; CSF, cerebrospinal fluid; Cx43, connexin 43; DHA, docosahexaenoic acid; DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; DCPIB, 4-(2-butyl-6,7-dichlor-2-cyclopentyl-indan-1-on-5-yl)-oxybutyric acid; GSK1016790A, N-((1S)-1-{[4-((2S)-2-{[(2,4-dichlorophenyl)sulfonyl]amino}-3-hydroxypropanoyl)-1-piperazinyl]carbonyl}-3-methylbutyl)-1-benzothiophene-2-carboxamide; HC-067047, 2-Methyl-1-[3-(4-morpholinyl)propyl]-5-phenyl-N-[3-(trifluoromethyl)phenyl]-1 h-pyrrole-3-carboxamide; EAATs, Na+-dependent excitatory amino acid transporters; GLUT, glucose Transporter; HOE642, cariporide; IMD-0354, N-(3,5-Bis-trifluoromethylphenyl)-5-chloro-2-hydroxybenzamide; Mfsd2a, major facilitator superfamily domain containing protein 2a; Kir4.1, inward rectifier potassium channel; LRRC8, leucine-rich repeat-containing 8; mGluR5, metabotropic glutamate receptor 5; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine hydrochloride; NAM, negative allosteric modulators; NBC, Na+-HCO3- cotransporter; NHE1, Na+/H+ exchanger 1; NKCC1, Na+-K+-2Cl- cotransporter 1; PA, potassium aspartate; PIP2, phosphatidylinositol 4,5-bisphosphate; RN-1734, 2,4-Dichloro-N-isopropyl-N-(2-isopropylaminoethyl)benzenesulfonamide; RN-1747, 4-Chloro-2-nitro-1-((4-benzylpiperazinyl)sulfonyl) benzene; SGLT1, sodium-glucose linked transporter 1; S0859, C29H24ClN3O3S; SUR1, sulfonylurea receptor 1; TFBTBOA, 3-[3-[4-(tri-fluoromethyl)benzoylamino]benzyloxy] aspartate; TGN-020, N-(1,3,4-thiadiazol-2-yl) pyridine-3-carboxamide dihydrochloride; TRPM4, transient receptor potential melastatin 4; TRPV4, transient receptor potential vanilloid 4; VRAC, volume-regulated anion channel; VU0360172, N-cyclobutyl-6-((3-fluorophenyl)ethynyl) nicotinamide hydrochloride; 4αPDD, 4alpha-phorbol 12,13-didecanoate.

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Disclosure

The authors report no conflicts of interest in this work.

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