REVIEW ARTICLE

Molecular Mechanism by Which TRPC6 Regulates Calcium Signaling and Neuroinflammation in the Onset and Development of Ischemic Stroke: A Review

Wenbin Li, PhD, Yidan Zhang, MM, Fan Yang, PhD, Lei Zhang, PhD

ABSTRACT

Cerebral infarction, also known as ischemic stroke, is caused by various regional blood supply disorders in the brain tissue, leading to ischemic hypoxic lesions and necrosis of the brain tissue and then the corresponding clinical manifestations of neurological loss, which has high mortality and disability. This study comprehensively reviews the potential molecular mechanisms of TRPC6 in neuroprotection in cerebral infarction and provides a summary of TRPC6 as a targeted drug or prognostic biomarker for cerebral infarction patients. We will screen and synthesize evidence about the molecular mechanisms of TRPC6 in cerebral infarction from the current literature to obtain comprehensive knowledge on this topic.

In the pathogenesis, neuroinflammation and intracellular calcium accumulation play an important role in the onset and development of cerebral infarction. Transient receptor potential cation channel subfamily C6 (TRPC6) is the main component of calcium store-operated calcium channels. It plays a central role in ischemic cerebrovascular disease by mediating the calcium ion signaling pathway. In this review, evidence on the neuroprotective effects of TRPC6 has been shown, including inhibiting neuroinflammation and inhibiting nerve cell apoptosis, thereby alleviating nerve injury. However, at the same time, TRPC6 promotes inflammation in other organs.

Generally, although an increasing number of researches support the protective role of TRPC6 in cerebral infarction, there is still evidence showing that overexpression of TRPC6 increases inflammatory tissue damage in other organs. Therefore, clarifying the molecular mechanism of TRPC6 will help develop targeted drugs or prognostic biomarkers for cerebral infarction to promote and predict neurological function recovery. More evidence to elucidate the molecular mechanism of TRPC6 in cerebral infarction is needed. Enriching TRPC6 in neuroinflammation areas and modifying its cell specificity might be the orientation of drug development that increases the effect of stroke treatment and reduces the impact on other organs.

In conclusion, in cerebral infarction, TRPC6 has been proven to alleviate neuroinflammation and inhibit nerve cell apoptosis. However, at the same time, TRPC6 may promote inflammation in other organs. Therefore, the targeting potential of TRPC6 in cerebral infarction needs to be further explored. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

Cerebral infarction, also known as ischemic stroke, is the most important type of stroke in clinical practice. This disease is caused by various reasons regional blood supply disorders in the brain tissue, leading to ischemic hypoxic lesions and necrosis of the brain tissue and then the corresponding clinical manifestations of neurological loss.[1] Stroke has become the leading cause of death and disability among the Chinese population, of which approximately 86.9% are ischemic strokes. It has been reported that the in-hospital mortality rate of stroke patients is approximately 8.9%-16.8%. The three-month disability rate among survivors is approximately 14.8%.[2] The pathological process of cerebral infarction is extremely complex, but the initiating stage is triggered by cerebral ischemia. Occlusion of the cerebral artery with thrombosis causes a decrease in brain blood perfusion, resulting in ischemia and hypoxia, softening and even necrosis of brain tissue. The infarct size of patients with

middle cerebral artery occlusion mainly depends on the collateral blood supply. A good collateral blood supply is an important prerequisite for limiting the volume of cerebral infarction and the success of spontaneous or drug-induced recanalization.[3] Some researchers have suggested that blood circulation should be restored in time in the treatment of cerebral infarction, and the volume of neuronal death caused by ischemia should be minimized.[4,5] Although reperfusion may cause damage, the benefits of reperfusion outweigh the disadvantages.[4] Thrombolytic therapy is the most effective way to slow nerve damage and accelerate cerebral function recovery in the treatment of cerebral infarction.[6] It is well known that the thrombolytic drug tPA (tissue plasminogen activator) is used for the clinical treatment of cerebral infarction. The FDAThe FDA has approved it has approved it, but tPA easily aggravates damage to the blood–brain barrier and induces cerebral hemorrhage.[7] In addition, various factors affect the process of neural repair in patients with cerebral infarction.[8] Evidence has shown that neuroinflammation plays an important role in the pathogenesis of cerebral infarction.

TRPC6, as a receptor-activated nonselective calcium-permeable cation channel, plays an important role in the neuroinflammatory response to cerebral infarction. We plan to synthesize the evidence about the molecular mechanisms of TRPC6 in cerebral infarction from the current literature to obtain comprehensive knowledge on this topic in terms of neuroinflammation, calcium signaling, and the involved cell signaling pathways. And then this study will provide a theoretical summary for TRPC6 as a drug target or biomarker for nerve injury.

The role of neuroinflammation in cerebral infarction

The role of reducing neuroinflammation in cerebral infarction treatment. Cerebral infarction is a highly complex and heterogeneous disease. Inflammation and thrombosis are two important pathophysiological cascades of cerebral infarction [9]. In particular, inflammation directly relates to many pathogenic processes of brain injury caused by cerebral infarction. After cerebral infarction, dead cells quickly release High-mobility group protein B1 (HMGB1) and other toxic components, which promotes the activation of immune cells, including microglia/macrophages and astrocytes, and triggers neuroinflammation.[10] At the same time, the intervention of cerebral ischemia-reperfusion injury (CIRI) is a key issue in the treatment of cerebral infarction. The pathophysiological mechanisms of CIRI include excitotoxic neurotransmitter release, intracellular calcium accumulation, free radical damage, neuronal apoptosis, neuroinflammation, etc.[11,12] Neuroinflammation plays a key role in the pathophysiological process of cerebral infarction and is currently considered to be an important target for the treatment of cerebral infarction.[13]

Microglia/macrophage activation promotes neuroinflammation. Microglia are immune cells in the central nervous system that are first activated after cerebral infarction. Importantly, the phenotype of activated microglia/macrophages and astrocytes determines the beneficial or detrimental effects of neuroinflammation after cerebral infarction.[14] After cerebral infarction, the destroyed blood–brain barrier and the chemokines secreted by immune cells at the brain injury site recruit and activate macrophages in the peripheral blood circulation in brain tissue.[10] Activated microglia are rod-shaped, spherical or amoeba-like in morphology and are difficult to distinguish from invading macrophages. Additionally, microglia and macrophages in the peripheral blood system both express biomarkers such as ionized calcium adapter molecule 1 (Ibal), CD68 and CD11b; therefore, both are often called microglia/macrophages.[15]

Activated microglia/macrophages are polarized into the M1 phenotype with a proinflammatory effect and the M2 phenotype with an anti-inflammatory effect after cerebral infarction. Microglia/macrophages of the M2 type limit toxic neuroinflammation caused by cerebral infarction, reduce brain tissue damage, and are beneficial to the recovery of neurological function.[16] Moreover, after cerebral infarction, astrocytes are also rapidly activated and secrete a variety of factors that promote or inhibit inflammation, which are involved in the regulation of neuroinflammation. Reactive astrocytes display two polarization states called A1- and A2-type astrocytes.[17] A1-type astrocytes secrete toxic factors, aggravate neuroinflammation and induce neuronal death, while A2-type astrocytes can inhibit toxic neuroinflammation and protect nerve cells.[18]

The role of Transient receptor potential cation channel subfamily C6 (TRPC6) in cerebral infarction

Cerebral infarction is a blood supply disorder in the local brain tissue area caused by various reasons, resulting in cerebral ischemia and hypoxia and manifesting clinically corresponding neurological deficits.[19] Ischemia activates brain-resident cells, including microglia, astrocytes, and endothelium. The increased level of endothelial cell adhesion molecules and inflammatory cytokines/chemokines, coupled with increased permeability of the blood-brain barrier, enables ischemia-induced infiltration and activation of peripheral immune cells.[20,21] As the central role of calcium ions is found in ischemic cerebrovascular disease, TRPC family proteins are abundantly expressed in neurons, and TRPC6 is the most common. Therefore, in the study of the pathological mechanism of cerebral ischemia, researchers began to pay attention to the TRPC protein family, which is closely related to changes in calcium ions in neurons.[22]

In general, the role and molecular mechanisms in the inflammatory response of cerebral infarction are mainly manifested in the activation of resident brain cells, including microglia, astrocytes and endothelial cells. Increased levels of endothelial cell adhesion molecules and inflammatory cytokines/chemokines are associated with nervous system inflammation, microglial activation, and the expression of inflammatory cytokines, chemokines, and key proteins such as Toll-like receptor 4 (TOR4), HMGB1, and TRPC family proteins.

TRPC6 mediates Ca^{2+} signaling in cerebral infarction

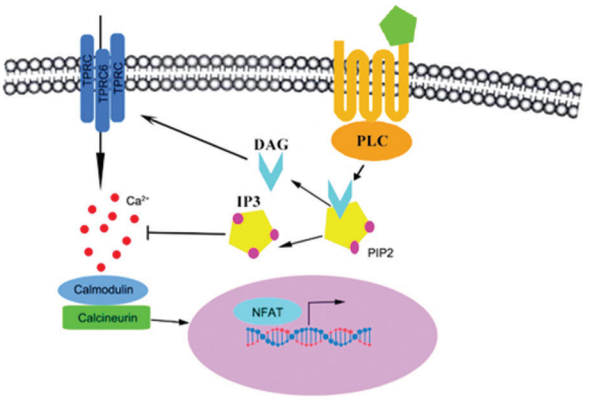
The mechanism of Ca^{2+} signaling involved in cerebral ischemia-reperfusion injury. In cerebral infarction and ischemia-reperfusion injury, Ca^{2+} signaling under the regulation of TRPC6 may be involved in the regulation of neuroinflammation and recovery of neurological function. Cerebral ischemia-reperfusion injury is a complex pathophysiological process. In the early stage, it is mainly caused by ischemia of the brain, and calcium-iron overload leads to reperfusion injury in the later stage.[23] Generally, the volume of cerebral infarction, neurological inflammation and activation of microglia, as well as the expression of inflammatory factors, chemokines and important key proteins such as TOR4, HMGB1, TRPC family, etc. Calcium ion (Ca^{2+}) is an important second messenger in cells and is involved in the regulation of various processes such as muscular cell contraction and relaxation, proliferation and differentiation, transmitter release and death. In eukaryotes, cells regulate Ca^{2+} concentration through various mechanisms, and store-operated calcium channels (SOCs) are the main channels for regulating the concentration of Ca^{2+} in nonexcited cells, which provides normal transmission of signals. After cerebral ischemia, calcium channels, as second messengers, are overopened, and the concentration of calcium in the brain is too high.[24]

Regulation of Ca^{2+} signaling by the TRPC family. The Na(+)/Ca($^{2+}$) exchanger (NCX) plays an important role in microglial function stimulated by pathological factors such as interferon-gamma or nitric oxide (NO) exposure.[25] Ca^{2+} channels may play a prominent role and provide a novel choice in neurological inflammation and neuronal cell death.[26] K/Ca^{2+} channel-mediated calcium homeostasis may preserve normal function and prevent excitotoxic neuronal death, which serves as a therapeutic target for reducing microglial activity and related inflammatory responses in the central nervous system.[27]

TRPC is involved in the composition of store-operated calcium entry (SOCE), which was originally found in Drosophila. It is a nonselective cation channel protein located on the cell membrane that selectively permeates sodium and calcium ions. There are seven subfamily members of TRPC, which can be further divided into two subclasses according to amino acid sequence homology and structural characteristics: TRPC1/2/4/5 and TRPC3/6/7, of which TRPC2 is not expressed in humans.[28] TRPC6 is involved in the occurrence and development of various diseases.[29,30] The mechanism may be that the TRPC6 gene promoter region contains the nuclear factor of activated T cells (NFAT) response element, which is involved in the increase in intracellular Ca^{2+} concentration, resulting in intracellular Ca^{2+} disorder, which mediates Ca^{2+} signaling, which in turn causes the activation of downstream disease-related target genes.[31-33]

Under normal circumstances, the stress of cells from the resting state to the activated state requires an increase in the concentration of cytoplasmic free Ca^{2+}, and the increase in Ca^{2+} concentration is related to the action of various Ca^{2+} channel proteins. The opening of TRPCs channels can initiate the influx of Ca^{2+} in cells and increase the concentration of Ca^{2+} in the cytoplasm, endoplasmic reticulum, and mitochondria.[34] Studies have shown that the increase in intracellular calcium levels in vascular smooth muscle cells is a key factor leading to vasoconstriction, and the increase in intracellular Ca^{2+} concentration occurs mainly through cell membrane voltage-dependent calcium channels (VDCCs) and receptor-gated calcium channels.[35] Receptor-operated calcium channels (ROCCs) cause a large influx of extracellular Ca^{2+}, and the influx of Ca^{2+} binds to calmodulin (CaM) to form a complex to activate myosin light chain kinase (myosin light chain kinase, MLCK), which further triggers smooth muscle contraction.[36,37] TRPC is the molecular basis for the formation of ROC and SOC on the cell membrane, and TRPC6 is mainly involved in the formation of ROCC. Stimulation leads to the opening of ROC and VOC channels, which provides a bulk flow of Ca^{2+} into cells in a short time. At the same time, SOC continues to produce a small amount of calcium influx to ensure the normal transmission of signals.[38,39] For example, agonists can activate phospholipase C (PLC) to hydrolyze phosphatidylinositol 4,5-diphosphate (PIP2) to form diglycerides (diacylglycerol, DAG) and inositol triphosphate (IP3). DAG can directly activate TRPC3/6/7 and increase intracellular Ca^{2+} influx. IP3 binds to receptors on the endoplasmic reticulum membrane, reduces the calcium concentration in the calcium pool, depletes the intracellular calcium pool, and activates the opening of TRPCs channels to allow Ca^{2+} influx (Figure 1). Luteolin has been shown to treat cerebral infarction by inhibiting MMP9 and activating the PI3K/Akt pathway.[40]

Figure 1. TRPC6 regulates the Ca^{2+} channel. Activated phospholipase C (PLC) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to form diglycerides (diacylglycerol, DAG) and inositol triphosphate (IP3). DAG directly activates TRPC6 to increase the influx of intracellular Ca^{2+}.



TRPC6-mediated signaling pathway in cerebral infarction and the inflammatory response

Not only through the Ca^{2+} signaling pathway, TRPC6 may also be involved in alleviating neuroinflammation through other potential signaling pathways. TRPC6 has been reported to

Figure 2. TRPC6-related signaling pathways in the inflammatory response. Various TRPC6-mediated signaling pathways are reported in the progression of cerebral infarction and inflammatory responses, including the PI3K/Akt pathway, MAPK pathway, NFAT pathway, CREB-related pathway and NF-kB pathway.

protect neurons from cerebral ischemic injury; for example, in a cerebral ischemia model, increasing TRPC6 activity inhibits neuronal death, but blocking TRPC6 increases sensitivity to ischemia.[34] Studies have shown that TRPC6 is abundantly expressed in rat cortical neurons and involved in neuronal apoptosis.[41] In addition, it has been reported that activating TRPC6 in neurons can phosphorylate cAMP-response element binding protein (CREB), thereby playing a protective role in neurons.[42] However, when cerebral ischemia-reperfusion injury is caused by cerebral infarction, abnormally activated calpain degrades TRPC6, aggravating cerebral lesions.[42] Moreover, our research found that overexpression of TRPC6 in bone marrow stromal cells (BMSCs) decreased cerebral ischemia/reperfusion injury.[43] Inhibiting the degradation of TRPC6 alleviated ischemic neuronal cell death in an animal model of cerebral infarction.[44,45] In a middle cerebral artery occlusion (MCAO) mouse model, the expression of TRPC6 was obviously decreased during I/R injury in vitro and in vivo.[44] Overexpression of astrocytic TRPC6 decreased inflammatory responses and NF-kappaB phosphorylation,[22] suggesting that TRPC6 might be a promising target to alleviate inflammatory responses in astrocytes during I/R injury and alleviate ischemic brain damage. TRPC6 suppresses the activity of NMDA receptors and protects neurons from ischemic excitotoxicity via the CREB signaling pathway.[22] Another study proved that inhibition of TRPC6 degradation suppressed ischemic brain damage in rats.[45] In summary, a number of studies have reported that TRPC6 plays a protective role in the progression of cerebral infarction.

Neuroinflammation is a double-edged sword. On the one hand, it aggravates neuronal injury; on the other hand, it promotes tissue repair and myelin regeneration.[46,47] Therefore, how to protect the blood-brain barrier, reduce the side effects of thrombolytic drugs, and improve the effectiveness of thrombolytic therapy needs further research. At the same time, studying the mechanism of neuroinflammation and finding new neuroinflammatory targets will contribute to the prevention and clinical treatment of cerebral infarction.[34]

However, in other organs, TRPC6 may exhibit a role in promoting inflammation. It seems that TRPC6 plays a completely different role in ischemia-reperfusion injury in brain tissue and other tissues, such as the kidney, liver, and myocardium.[22,48] Various TRPC6-mediated signaling pathways are reported in the progression of cerebral infarction and inflammatory responses (Figure 2).

In addition to being involved in the development of cerebral infarction, TRPC6 has also been confirmed to be involved in inflammatory responses in other organs, but may play a role in promoting inflammation. In research on liver dysfunction and fibrosis, TRPC3 or TRPC6 gene-deficient (KO) mice showed no significant difference in inflammation compared with wild-type (WT) mice.[49] Treatment with O_3 or H_2O_2 increased TRPC6 levels in vivo and in vitro, which regulated the TRPC6-mediated Ca^{2+} pathway, leading to the activation of the ERK pathway and the inflammatory response, and both TRPC6(-/-) mice and mice pretreated with SAR7334, a potent TRPC6 inhibitor, alleviated the inflammatory response induced by O_3.[50] Hyp9, an activator of TRPC6, induces the production of cytokines, including IL-8 and IL-6.[51] Additionally, LPS promoted the expression of TRPC6 and Ca^{2+} influx through the TLR4/PI3K/AKT signaling pathway and subsequently activated the inflammatory response by the ERK1/2, p38, and NF-kappaB pathways.[51] TRPC6 was increased in the kidneys of patients with diabetic nephropathy (DN) and associated with tubular injury and inflammation, and tacrolimus (TAC) ameliorated tubulointerstitial inflammation in DN through the NFATc1/TRPC6 feedback loop.[52] Another study found that TRPC6 expression was significantly upregulated in DN tissues and cells and that overexpression of TRPC6 promoted the release of IL-8 and IL-6 in a rat model of DN.[53] Leukocyte transendothelial migration (TEM) is critical to the inflammatory response, which requires a transient increase in endothelial cytosolic free calcium ion concentration.[54] TRPC6, as a Ca^{2+} channel expressed in endothelial cells, interacts with PECAM and plays an important role in TEM and inflammation.[54,55] Additionally, TRPC6 does not play a role in the kidney damage of acute ischemic kidney injury.[56,57]

Clinical relevance and future research

Mechanistically, TRPC6 may help to reduce neuroinflammation and inhibit nerve cell apoptosis and therefore can be used as an indicator to predict neurological function recovery after IS. Patients with high expression of TRPC6 may have better neurological recovery. In further research, clinical studies can be performed to confirm the prediction accuracy of TRPC6. TRPC6 also has the potential to be a targeted drug to promote neurological function recovery after cerebral infarction. In further research, through appropriate administration routes and changing the cell specificity, TRPC6 can be used to treat IS with fewer adverse effects on other organs.

CONCLUSION

Overall, although an increasing number of studies support the protective role of TRPC6 in cerebral infarction, there are still studies showing that overexpression of TRPC6

increases inflammatory tissue damage in other organs, such as the liver, lung, and kidney. A comprehensive understanding of TRPC6 will help to develop targeted drugs to help inhibit neuroinflammation and nerve cell apoptosis after stroke to promote the recovery of neurological function. At the same time, TRPC6 can also be used as a biomarker to predict neurological function recovery after cerebral infarction. For example, TRPC6 expression levels in cerebrospinal fluid can more accurately predict neurological recovery than those in peripheral circulation. Supplementing exogenous TRPC6 in cerebrospinal fluid may be more beneficial to neurological recovery than peripheral administration.

The methodological variations and animal model differences may have contributed to the contradictory findings. More research evidence is needed to confirm the molecular mechanism of TRPC6 in cerebral infarction. In addition, in the state of cerebral infarction disease, the mechanism of TRPC6 in other organs also needs to be confirmed.

In summary, TRPC6 has the potential to be a prognostic biomarker and targeted protein for neurological function recovery. However, there is still an enormous gap between the current research evidence and clinical application.

DATA AVAILABILITY

The simulation experiment data used to support the findings of this study are available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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AUTHOR CONTRIBUTIONS

Wenbin Li, PhD and Yidan Zhang, MM contributed equally.

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