Introduction

Stroke is one of the leading causes of death and disability in the world[1, 2]. The disease itself and associated morbidity have caused significant social and economic impacts on society and individuals worldwide. The prevalence of stroke is expected to increase and our aging population is especially vulnerable to stroke insults. The clinical trials of anti-excitotoxic therapies (AET) have failed to benefit stroke patients[3], thus diminishing the initial excitement of translating research from bench to bedside and using glutamate receptor blockers in treating stroke patients. Even though the mechanisms underlying cerebral ischemia are beginning to be better understood, there is still no clinical or experimental treatment that has shown improved outcome for stroke patients. To ease personal and societal burden of stroke, continuous efforts have been directed towards searching for new therapeutic targets in stroke. This review provides a current view on one of the non-glutamate mechanisms of stroke that mediates through TRPM7 channels from a recent in vivo study[4].

A major event during cerebral ischemia is a concomitant massive release of the excitatory neurotransmitter glutamate, which results in intracellular calcium overload and eventual cell death[5]. The excitotoxicity in ischemia has been in the centre of stroke research for a long period of time. Triggered release of excessive glutamate causes cell death following ischemia, which is associated with an increase of the intracellular calcium (Ca2+) concentration[6–8]. Thus, identifying the source of the excessive Ca2+ influx and/or release from the intracellular Ca2+ stores during ischemia has been a research focus. Traditionally, Ca2+-permeable NMDA (N-methyl-D-aspartic acid), AMPA (DL-alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors[8] and L-type voltage-dependent Ca2+ channels[9] were considered as the major calcium entry paths and the causes of Ca2+ overload during ischemia. This Ca2+ overload, or a broad spectrum of ion imbalance, during ischemia is considered to initiate a wide range of sequential events that lead to irreversible damage to protein synthesis, mitochondria, cytoskeleton and plasma membrane, and to eventual cell death. In an ideal scenario, the interruption of this Ca2+ overload in ischemia is thought to be clinically beneficial for stroke patients. Blocking these receptor channels prevents the intracellular Ca2+ overload and provides significant neuroprotection in the laboratory. Some of the findings from the bench have been translated into many clinical trials in stroke treatment. However, the results of clinical trials testing AET, which include NMDA and AMPA receptor block-

TRPM7 in cerebral ischemia and potential target for drug development in stroke

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Searching for effective pharmacological agents for stroke treatment has largely been unsuccessful. Despite initial excitement, antagonists for glutamate receptors, the most studied receptor channels in ischemic stroke, have shown insufficient neuroprotective effects in clinical trials. Outside the traditional glutamate-mediated excitotoxicity, recent evidence suggests few non-glutamate mechanisms, which may also cause ionic imbalance and cell death in cerebral ischemia. Transient receptor potential melastatin 7 (TRPM7) is a Ca2+ permeable, non-selective cation channel that has recently gained attention as a potential cation influx pathway involved in ischemic events. Compelling new evidence from an in vivo study demonstrated that suppression of TRPM7 channels in adult rat brain in vivo using virally mediated gene silencing approach reduced delayed neuronal cell death and preserved neuronal functions in global cerebral ischemia. In this review, we will discuss the current understanding of the role of TRPM7 channels in physiology and pathophysiology as well as its therapeutic potential in stroke.

Keywords: ion channels; TRP; TRPM7; cerebral ischemia; stroke; in vivo test; siRNA; neuroprotection

Acta Pharmacologica Sinica (2011) 32: 725–733; doi: 10.1038/aps.2011.60; published online 9 May 2011

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Received 2011-03-14 Accepted 2011-04-18
ers, turned out to be ineffective and even with unwanted side effects\cite{10-14}. This may be due to multiple factors and it will not be the focus of this review\cite{15}. Because of the limitation of the glutamate mechanism and the unfavourable outcomes of AET trials, stroke researchers have been seeking for alternative, non-glutamate related therapeutic targets that cause ionic imbalance and cell death. Some of these channels include: acid-sensing ion channels\cite{16,17}, transient receptor potential (TRP) channels\cite{4,7,18-21}, and hemichannels\cite{22-24}, volume-regulated anion channels\cite{25}, sodium-calcium exchangers\cite{26,27} and non-selective cation channels\cite{28}.

Based on the recommendations from the Stroke Therapy Academic Industry Roundtable (STAIR) committee, it is important to validate the preclinical development in proof of concept starting with in vivo rodent models as experimental animal stroke models\cite{29}. Recent in vivo studies aimed at identifying the non-glutamate mechanisms for stroke have demonstrated the involvement of acid-sensing ion channels\cite{16,17} first, and then the TRPM7 (transient receptor potential melanostatin 7) channel\cite{18-21}. In this review, we will mainly focus on the current understanding of the molecular, biological, and pharmacological properties of TRPM7 as well as its physiological and pathophysiological roles and its therapeutic potential in stroke.

**Classification, structures and distributions**

**Classification**

The TRP superfamily is comprised of a group of non-selective cation channels\cite{30-33}. Its nomenclature was originated from the first found member of this superfamily, which was identified in a *Drosophila* phototransduction mutant showing transient receptor potential to a continuous light\cite{34}. Currently, about 30 mammalian TRP channels have been discovered and named according to their sequence homologous structures. They are classified into six subfamilies: 1) TRPC (canonical), 2) TRPM (melastatin), 3) TRPV (vanilloid), 4) TRPA (ankyrin), 5) TRPML (mucolipin) and 6) TRPP (polycystin). Different TRP channels are activated by different physical and chemical stimuli. The diverse gating mechanisms of TRP channels make them good cellular signal integrators critical for physiological and pathological functions\cite{30-33}.

TRPM7 belongs to the melastatin-related subfamily of TRP channels, which is comprised of eight members (eg, TRPM1-8). It was suggested that TRPM7 may also form heteromers with TRPM2 as application of TRPM7 siRNA also down-regulated TRPM2 channel mRNA in an *in vitro* study\cite{21}. This is important as TRPM2 has also shown to play a role in oxidative stress-mediated cell death, which is a cellular condition shown in stroke.

**Gene and protein structures**

In human, TRPM7 gene is located on chromosome 15 in the q21.2 region, and encoded by 39 exons that spans about 127 kb of DNA sequence. The mouse TRPM7 gene is 95% identical to human gene\cite{30}. It is located on chromosome 2 on cytoband F2 and it is also encoded by 39 exons that spans about 85 kb of DNA sequence.

TRPM7 is a large protein (1864 amino acids in human; 1863 amino acids in mouse) with a predicted molecular weight of approximately 212 kDa. Each subunit has six transmembrane (TM) spanning domains (S1-S6) with a re-entrant pore-forming loop (known as P-loop) between the fifth (S5) and sixth (S6) segments\cite{32,33} (Figure 1). The N-terminus has another hydrophobic region (H1) and four regions of TRPM subfamily homology domain (MHD), but their biological significance is largely undefined. The C-terminus contains a TRP box of ~25 highly conserved residues, which may interact with phosphorylidyinositol 4,5-bisphosphate (PIP2), a positive regulator of some TRP channel\cite{36}. A coiled-coil domain close to the C-terminus may mediate subunit-subunit interactions and tetrameric assembly of TRPM7\cite{37}. The most unique structural feature of the channel is the enzymatic domain located at the end of C-terminus. In TRPM7, the distal C-terminus has an atypical serine/threonine protein kinase domain that is homologous to a family of α-kinases\cite{38}. Although this kinase domain does not seem to affect channel activity directly\cite{33,39,40}, it may be important for the regulation of channel function by Mg2+ nucleotides\cite{41}.

**Tissue and cellular distribution**

TRPM7 channel mRNA is ubiquitously expressed in almost all tissues\cite{30,42,43}. Recently, real-time quantitative RT-PCR analyses with either Taqman or SYBR Green were used to create comparative distribution profiles of TRPM channels in selected human tissues, including brain, pituitary, heart, lung, liver, fetal liver, skeletal muscle, stomach, intestine, spleen, peripheral blood mononuclear cells, macrophages, pancreas, prostate, placenta, cartilage, bone and bone marrow\cite{42}. TRPM7 mRNA has the highest expression in heart, pituitary, bone, and adipose tissue\cite{42}. Similar distribution patterns of TRPM7 were also observed in mouse tissue samples\cite{44}. Compared to other TRP members, TRPM7 mRNA expression levels were significantly higher in most tissues.

The TRPM7 protein shown by immunofluorescent labeling is strongly expressed at the plasma membrane in N1E-115 neuroblastoma cells\cite{45}, and in vascular smooth muscle cells\cite{46}. In N1E-115 neuroblastoma cells, HA-tagged TRPM7 antibodies were localized in membrane ruffles\cite{46}. Similarly, protein expression of TRPM7 is also shown in cell bodies and processes of hippocampal neurons with immunostaining\cite{44,47,48}. In superior cervical ganglion neurons, TRPM7 is exclusively localized within cholinergic vesicles\cite{49}.

**Biophysical properties, regulatory mechanisms, pharmacology**

**Biophysical properties**

TRPM7 is a non-selective cation channel that displays several biophysical features that make this channel distinguishable from other TRP members. TRPM7 channel has a reversal potential of approximately 0 mV, and a prominent outward rectification\cite{30,33,43,50} (Figure 1B). At negative membrane potentials, TRPM7 conducts a small inward current by trans-
porting divalent cations (e.g., calcium and magnesium) down their concentration gradients. TRPM7 current density is usually under 20 pA/pF [35, 50]. At positive membrane potential, TRPM7 conducts a strong outward current as intracellular cations experience strong driving force to exit the cell. This outwardly rectifying property is entirely due to a voltage-dependent block of monovalent cations by extracellular divalents. For instance, in the absence of divalent cations, TRPM7 conducts inward monovalent cations [35]. Consequently, its I-V relation becomes quasi-linear suggesting the lack of voltage-dependent gating and channel selectivity (Figure 1B).

Unlike many other Ca²⁺ permeating channels, TRPM7 is characteristically more permeable to a series of trace metal ions. Using equimolar divalent ion substitution approaches, Monteilh-Zoller and colleagues reported a permeation profile for TRPM7 in a sequence of: Zn²⁺ ≈ Ni²⁺ >> Ba²⁺ > Co²⁺ > Mg²⁺ ≥ Mn²⁺ ≥ Sr²⁺ ≥ Cd²⁺ ≥ Ca²⁺ [51]. TRPM7 allows entry of these divalent ions even with physiological levels of extracellular Ca²⁺ and Mg²⁺. TRPM7 is constitutively active and this feature makes TRPM7 a good candidate for both sensing the extracellular concentration of divalents and maintaining intracellular Mg²⁺ homeostasis during ischemic episodes that lead to intense neuronal activity [47].

TRPM7 channel activity is regulated by extracellular pH. A decrease in extracellular pH (acidic) strongly potentiated current activity of the recombinant TRPM7 channel expressed in HEK-293 cells (~10-fold increase at pH 4.0, and 1-2 fold increase at pH 6.0) [52] and in CHOK1 cells (~12-fold increase at pH 4.0) [53]. However, the TRPM7-like current in the FaDu cell line was insensitive to the acidic condition (pH 5.0) [54], while the TRPM7-like inward current in human cerebral epithelial HeLa cells was increased at pH 4.0 [55]. Protons likely compete with Ca²⁺ and Mg²⁺ for their binding sites, thus increase the inward current by releasing the divalent cation block [52]. Point mutation of Glu 1047 (E1047Q) of TRPM7 channels eliminated the proton-enhanced inward current activity, indicating the residue may be involved in the pH sensitivity of the channels [53]. Although the effects of protons on endogenous TRPM7 remains controversial, TRPM7 can be regulated in acidic pathophysiological conditions, including ischemic stroke [56].

TRPM7 is not a mechanosensitive channel, however, shear stress in vascular smooth muscle cells doubled the number of TRPM7 channels near the plasma membrane [57]. Further studies are required to deduce the mechanism of stress-induced regulation of TRPM7 channels.

Figure 1. Schematic diagram showing proposed transmembrane topology of TRPM7. (A) The putative membrane topology of a single subunit of TRPM7 is shown. Each subunit has six transmembrane (TM) spanning domains (S1–S6) with a re-entrant pore-forming loop between the fifth (S5) and sixth (S6) segments. The intracellularly located N-terminus has another hydrophobic region (H1) and four regions of TRPM subfamily homology domain (MHD). The intracellularly located C-terminus contains a TRP box of ~25 highly conserved residues (TRP) and a coiled-coil domain (CCD). The distal C-terminus has an atypical serine/threonine protein kinase domain. As indicated in the figure, TRPM7 is a non-selective cation channel that conducts both monovalent ions (e.g., Na⁺ and K⁺) and divalent ions (e.g., Ca²⁺, Mg²⁺ and other trace metal ions). (B) Representative current-voltage (I-V) relationship of TRPM7.
Whole-cell patch-clamp recordings have shown that either adding Mg2+ chelators (e.g. HEDTA or Na-ATP) intracellularly or omitting Mg2+ and Mg2+-complexed nucleotides in intracellular solutions increased activation of TRPM7[40, 41, 43]. These inhibitory effects may be mediated by binding to C-terminal kinase domain[39, 43], which in itself is not essential for the activation of TRPM7[39, 39, 43]. Compared to the wild-type, phosphotransferase-deficient mutant channels (K1648R and G1799D) demonstrated a reduced sensitivity to inhibition by Mg2+ at intermediate concentrations close to the IC50[39]. Moreover, Demeuse and colleagues[41] reported differential sensitivity to Mg2+ and Mg2+-nucleotides inhibition in the wild-type, phosphotransferase deficient point mutant (K1648R), and the Δ-kinase truncation mutant. These findings lead to a hypothetical model: only Mg2+-nucleotides bind to the kinase domain but this domain interacts with the Mg2+-binding site, which is responsible for regulating the channel activity.

Activation of TRPM7 can be regulated via PIP2, which is a substrate of phospholipase C (PLC)[39]. The C2 domain of PLC is directly associated with the kinase domain of TRPM7. When carbachol, an agonist for G-protein coupled receptors, was used, PLC-beta was activated. This activation of PLC-beta led to the hydrolysis of localized PIP2, which caused a rapid decreased ITRPM7.

TRPM7 may also be regulated by phosphorylation. A variant of TRPM7 with a missense mutation (T1482I) is found in a subset of patients with Guamanian amyotrophic lateral sclerosis (ALS-G) and Parkinsonism-dementia (PD-G)[59]. The C2 domain of PLC is directly associated with the kinase domain of TRPM7. When carbachol, an agonist for Gαq-linked muscarinic type 1 (M1) receptors, was used, PLC-beta was activated. This activation of PLC-beta led to the hydrolysis of localized PIP2, which caused a rapid decreased ITRPM7.

Physiological functions

Our current knowledge of the physiological functions of TRPM7 channels has recently been improved, even with limited molecular and specific pharmacological tools. Under physiological conditions, several lines of evidence suggest the role of TRPM7 in cell survival and proliferation[39, 62, 72]. The early embryonic lethality in global TRPM7 knockout mice hints at the requirement of TRPM7 in cell survival and proliferation as embryonic development involves extensive cell proliferation[62]. In the same study, TRPM7 gene was selectively deleted in developing thymocytes. These T-cells did not differ in its ability of uptake Mg2+ or maintaining global cellular Mg2+, but showed defective thymopoiesis. A more recent study showed that knocking out of TRPM7 kinase domain homozygously resulted in embryonic lethality[63], while heterozygous knockout mice were viable, but exhibited abnormal homeostasis. TRPM7 knockout in chicken DT40 B cells caused growth arrest and eventual cell death in culture[39], which may be linked to a regulation of Mg2+ homeostasis[72]. Supplementing TRPM7 knockout cells with a high Mg2+ containing medium, but not Ca2+ or Zn2+, could restore normal cell growth and survival in culture. Knockdown of TRPM7 with RNA interference reduced Ca2+ and Mg2+ influxes, and decreased cell proliferation in human osteoblast-like cells[72], and retinoblastoma cells[73]. TRPM7 dependence for proliferation and differentiation was also shown in zebrafish mutants as they displayed severe growth retardation and general alterations in skeleton development[74].

Several studies have shown the importance of TRPM7 in cell adhesion. Over-expression of TRPM7 in HEK-293 cells lead to cell rounding, loss of adhesion and cell death[50]. Consis-
tent with these findings, knockdown of TRPM7 in HEK-293 cells increased cell adhesion[39]. Over-expression of TRPM7 may produce cell rounding by stimulating the activity of the Ca\(^{2+}\)-dependent protease m-calpain. TRPM7 has also been implicated in cell motility[78]. Knockdown of TRPM7 by RNA interference reduced the number of high Ca\(^{2+}\) micro-domains induced by platelet-deprived growth factor (PDGF) and disrupted the turning of migrating WI-38 fibroblasts. It has also been suggested that TRPM7 is involved in the neurotransmitter release by mediating Ca\(^{2+}\) influx[49]. In primary rat superior cervical ganglion neurons, TRPM7 is localized in the synaptic vesicles and interacts with synaptic vesicular syntaxin, synapsin 1 and synaptotagmin 1. Furthermore, there were some correlations between TRPM7 expression levels and quantal sizes, amplitudes and decay times of the excitatory postsynaptic potential (EPSPs). When TRPM7 specific siRNA was used to suppress endogenous TRPM7 in PC12 cells, acetylcholine-secreted-synaptic-like vesicle fusion was inhibited.

Pathophysiological relevance in cerebral ischemia and stroke
Unregulated monovalent or divalent cation influx is implicated in several different cellular mechanisms (e.g., excitotoxicity, apoptosis, and oxidative stress) underlying neuronal cell death during ischemic periods of stroke[61]. Since cation channels are the main pathways for cation influx from extracellular space, they are closely involved in neuronal cell death. Conventionally, Ca\(^{2+}\)-permeable NMDA and AMPA receptor channels are widely accepted as the main pathways of Ca\(^{2+}\) entrance during ischemia as well as the promising therapeutic targets[7, 8, 77]. Numerous clinical trials testing AETs in stroke patients, however, yielded disappointing outcomes. The shortcomings of AET led researchers to consider other non-glutamate dependent mechanisms[10–14], such as non-specific cation channels including acid-sensing ion channels[16, 17], TRP channels[4, 7, 18–21], hemichannels[1, 22–26], volume-regulated anion channels[27], sodium-calciunm exchangers[28] and non-selective cation channels[29].

Previous studies demonstrated pathophysiological involvement of TRPM7 in stroke from in vitro data. When primary cultured cortical neurons were subjected to oxygen-glucose anoxia was reduced. Such effects were consistently shown with cocktail of blockers for glutamate NMDA and AMPA receptor and L-type calcium channels (MK-801, CNQX, and nimodipine), indicating the independent role of TRPM7 in mediating intracellular Ca\(^{2+}\) elevation and subsequent cell death during the prolonged anoxia. In another study, the contribution of TRPM7 channels in cell membrane depolarization, intracellular Ca\(^{2+}\) accumulation and cell swelling during the initial period of brain ischemia is also observed in native CA1 neurons of brain slices[30].

TRPM7 in vivo studies have been scarce for a period of time because both the knockout model and selective pharmacological agents are not available. Eventually, a report demonstrated in vivo changes in TRPM7 channels during focal ischemia. Jiang and colleagues[48] studied the interaction of nerve growth factor (NGF) with TRPM7 channels using both in vivo cerebral ischemia-reperfusion and in vitro OGD models. NGF, a neurotrophic factor, showed neuroprotective effects during ischemia. In their in vivo model, middle cerebral artery occlusion (MCAO) was performed on rats for 1 h and it was followed by reperfusion that lasted for 5, 10, 20, and 30 h. Both mRNA and protein levels of TRPM7 were up-regulated compared with pre-ischemia, peaking at 20 h after the reperfusion with about 2–3 fold increase. Given that there are increases in both mRNA and protein levels of TRPM7, up-regulation of the channels may be another mechanism that increases TRPM7-like current. Interestingly, these expression levels of TRPM7 were close to the normal level when 500 ng of NGF was applied 30 min before ischemia. The effects of NGF on TRPM7, however, disappeared when NGF was introduced after K252a, which is an inhibitor for the NGF-activated TrkA pathway. When wortmannin, which is an inhibitor for phosphatidylinositol-3 kinase (PI-3K) signal pathway, was applied, NGF effects were also abolished. These findings indicate that TRPM7 may be involved in neuronal cell damage in vivo during ischemia.

Recently, Sun and colleagues[41] demonstrated that suppression of TRPM7 channels in vivo reduced neuronal cell death and preserved functions after global cerebral ischemia. The study used virally mediated gene silencing with shRNA to knockdown TRPM7 channels in hippocampal CA1 pyramidal neurons of adult rat brains. The viral vectors were delivered in vivo using stereotaxic microinjection to CA1 area. First, the authors showed that infecting adult hippocampal CA1 neurons in vivo was feasible by using the adeno-associated viral vectors (AAV serotype-1). Secondly, suppression of TRPM7 channels was convincingly demonstrated by measuring: 1) mRNA level in conjunction with the Laser Capture Microdissection for infected hippocampal CA1 cells; 2) protein level with both Western Blot and immunohistochemistry in conjunction with Laser Confocal microscope; and 3) functional level with electrophysiology. Thirdly, the injected viral vectors and transient suppression of TRPM7 channels in the adult rat brains in vivo showed no ill effects on cell survival, neuronal and dendritic morphology, neuronal excitability, or synaptic plasticity. Finally, they showed that following fifteen minutes of global cerebral ischemia induced by occluding both common carotid and vertebral arteries, TRPM7 suppression reduced hippocampal CA1 neuronal death in vivo and preserved functional outcomes after stroke. The survived neurons preserved their morphological integrity and fine structures, and even maintained their electrophysiological properties (LTP) and hippocampal-dependent behaviours, such as fear-associated and spatial-navigation memory tasks. This is
Working model of TRPM7 activation during cerebral ischemia

During the initial phase of an ischemic attack, a strong NMDA receptor activation leads to a large influx of Ca\(^{2+}\), and the resulting Ca\(^{2+}\) directly stimulates (i) production of nitric oxide (NO) by neuronal nitric oxide synthase (NOS) and (ii) production of superoxide (O\(_2^-\)) from mitochondria\(^{[17-19]}\). When NO and O\(_2^-\) are combined, highly reactive species peroxynitrite (ONOO\(^-\)) form. Along with other factors, such as decreases in pH and extracellular divalents, that are associated with ischemic episodes, ONOO\(^-\) enhances TRPM7 activation. This completes the lethal positive feedback loop of free radical production. In this model, the failure of AET could be partly explained: AET could delay the process but insufficient to ultimately prevent lethal TRPM7 activation (Figure 2).

TRPM7 channels may also be involved in the ischemic lethal process by conducting metal ions other than Ca\(^{2+}\). For instance, Zn\(^{2+}\), which is the most permeable trace ion through TRPM7, is highly toxic to cells if its concentration exceeds the physiological level\(^{[79, 80]}\) and has been implicated in cerebral ischemia. After the brief global ischemic insults, a delayed increase in intracellular Zn\(^{2+}\) is observed before cell death in some selective hippocampal CA1 neurons\(^{[81]}\). Increases in intracellular Zn\(^{2+}\) and neuronal cell death were prevented with the application of the membrane-impermeable zinc-chelator calcium-EDTA (calcium-ethylenediaminetetraacetic acid) before the ischemia. Recently, it has been shown that Zn\(^{2+}\)-induced neurotoxicity may be mediated by TRPM7\(^{[82]}\). Both Zn\(^{2+}\)-mediated neurotoxicity and neuronal injury associated with oxygen-glucose deprivation (OGD) were reduced by non-specific blockers (Gd\(^{3+}\) and 2-APB) and knockdown of TRPM7 by siRNA. Overexpression of TRPM7 in HEK-293 cells led to increase in intracellular Zn\(^{2+}\) accumulation and Zn\(^{2+}\)-mediated cell deaths.

Clinical potentials and therapeutic perspectives

To date, therapeutic intervention for stroke is very scarce. The only approved treatment of acute ischemic stroke by the US Food and Drug Administration (FDA) is the tissue plasminogen activator (tPA), which relieves vascular occlusion by dissolving clots\(^{[83]}\). Although tPA is a potent treatment for stroke, the usage and effectiveness of tPA are still limited by its short therapeutic window, and intrinsic toxicity. With disappointing preliminary clinical results from drugs targeting glutamate-induced excitotoxicity, considerable efforts have been put into searching for alternative targets.

Several lines of evidence support our hypothesis that TRPM7 is involved in ischemic stroke\(^{[27]}\). Even though findings from cellular and animal studies are compelling, TRP channels should be studied in their native cellular environment, as the specific cellular environment and expression levels seem to be important for the normal physiological functions. It would be necessary to validate their diverse physiological and pathophysiological functions using in vivo animal models. For in vivo studies, developing tissue-specific or inducible TRPM7 knockout models will be useful as the conventional TRPM7 knockout mouse is not viable\(^{[62, 63]}\).

Without the development of specific pharmacological modulators of TRPM7, we do not expect to see any preliminary clinical trials in the near future. At the initial stage of most drug development, potential therapeutic targets are first identified, and experimental high-throughput screening (HTS) is used to narrow down the drug candidates that bind to the targets and changes their activities\(^{[84]}\). Hence, in order to design specific and potent inhibitors, it is important to understand their molecular or structural properties in detail\(^{[84, 85]}\). This is especially true for TRPM7, which seems to have conflicting roles in cell death and cellular survival\(^{[16, 21, 62]}\.). It may be the case that TRPM7 function would have to be regulated sepa-
rately, either enhanced or depressed, in different tissues for the prevention of cerebral ischemia and stroke. For instance, Touyz and colleagues[86] have shown that reduced Mg$^{2+}$ influx in cultured vascular smooth muscle cells (VSMCs) of the spontaneously hypertensive rat (SHR) is associated with down-regulation of TRPM7. Furthermore in the normotensive Wistar-Kyoto rat, TRPM7 expression and activity in VSMCs of the SHR were attenuated by angiotensin II. Since hypertension is a well-known risk factor for cerebral ischemia and stroke, it suggests that TRPM7 channels may enhance cerebral ischemia and stroke by regulation of Mg$^{2+}$ homeostasis[87]. However, either supplement or depletion of Mg$^{2+}$ showed no effect on hypertension, thus questioning the role of TRPM7 in vasculature regulation[87]. In contrast, reduction of TRPM7 expression prevented cerebral ischemia and stroke[84] indicating TRPM7 may play a differential role in vascular smooth muscle cells and neurons. Further investigation is required to evaluate pathophysiological roles of TRPM7 channels in different cell types. Therefore, designing activity-dependent antagonists that preferentially target TRPM7 during stroke is critical in the future study. Understanding its temporal, spatial expressions and interactions with other proteins may aid the development of selective drugs for modulating TRPM7 activity.

When specific TRPM7 channel modulators are developed, these might be added in combination therapy for cerebral ischemic stroke in future. Now it is suspected that, although the recruitment of NMDARs is the key event in the early phase of cell death cascades in cerebral ischemia, there is also progressive recruitment of other non-selective cation channels, such as TRPM7, in the later stages. Based on this hypothesis, combination therapy, which includes drugs that are applied at empirically determined time points for each target, will be more effective in providing neuroprotection and potentially facilitating the recovery of function.

TRPM7 genetic variants may be related to various human diseases. For instance, heterologously expressed T1482I TRPM7 variant was found in a subset of ALS-G (Guamanian amyotrophic lateral sclerosis) and PD-G (Parkinsonism dementia) patients[59]. The mutation resulted in an increase in sensitivity of the channels to intracellular Mg$^{2+}$-mediated inhibition, thus the patients were more vulnerable to the diseases[59]. No test has been performed related to the risk assessment of ischemic stroke. Romero and colleagues[88], recently conducted a prospective, nested case-control investigation to evaluate the associations of TRPM7 gene variations with the risk of ischemic stroke, and showed that 16 tag-single-nucleotide TRPM7 polymorphisms from 259 Caucasian men had no direct association with the risk assessment of ischemic stroke. However, no test has been done to suggest whether these TRPM7 mutants have either dysfunction or abnormality of the expression level of the channels. Thus, these human studies lead to no conclusion between TRPM7 activation and cerebral stroke. Further study is needed to fully understand the biophysical properties of the TRPM7 polymorphisms. Such information will dramatically contribute to our current understanding of the pathophysiological role of TRPM7 in cerebral ischemia and stroke.

**Conclusions**

With the failure of using NMDA and AMPA antagonists in clinical trials for stroke treatment, other non-glutamate mechanisms to ischemic cell death have been rigorously investigated. Such disappointing clinical outcomes may originate from the insufficient understanding of non-glutamate mechanisms and their molecular cascades involved in stroke, problems in drug development, delivery of drugs, side effects of drug, or limited time windows for treatment. While the compelling findings from both in vitro and in vivo studies indicate the involvement of TRPM7 channels in ischemic neuronal injury, further extensive preclinical testing is required to assess the therapeutic potential of the TRPM7 blockade in stroke.

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