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TRPA1: A NEW FRONTIER IN ISCHEMIA / REPERFUSION TARGETING

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ABSTRACT

Ischemic conditions such as myocardial infarction, brain ischemia, and the peripheral vascular problems are primary causes of severe illnesses and fatalities in Western societies. These conditions arise from reduced blood flow to tissues. While reperfusion is essential for restoring the oxygen to these tissues, it paradoxically triggers harmful responses. These include the production of reactive oxygen species (ROS), inflammation among affected organs, stress of endoplasmic reticulum, and capillary no – reflow development after ischemia, exacerbating damage to the organ. Various pathological mechanisms play a role in ischemia / reperfusion injury, suggesting that targeting multiple pathways could lead to effective treatments. The Transient Receptor Potential A1 (TRPA1) channel, part of TRP ion channel group, responds to a wide array of chemicals and is involved in transmitting painful stimuli, including the effects of methylglyocal, ROS, and acrolein. This sensitivity is due to its response to changes in intracellular levels of calcium or phosphoinositol phosphate. Both ischemia and hypoxia, which are linked to stress in oxidation, activate TRPA1 channel. Current review focuses on role of TRPA1 and its associated processes/mechanisms in ischemia / perfusion. The evidence suggests that TRPA1 can either protect or worsen the condition during ischemia / reperfusion, depending on factors like its level of activation, affected area of ischemia, severity of the lesions, and duration of the ischemic events.

Keywords: TRPA1, Ischemia, Reperfusion, Therapy, Inflammation, Reactive oxygen species

Introduction

Ischemic events occurs due to an interruption in blood flow to specific tissues, resulting in a reduction of oxygen supply to these areas. This deficiency is addressed through reperfusion, which aims to reoxygenate the affected organs[1]. Organs that depend heavily on oxygen, such as the brain, liver, heart and kidney, and particularly vulnerable to ischemic events. Thus, ischemia / reperfusion (I/R) is a critical factor in the onset of various health conditions, including myocardial infarction, renal ischemia, diseases affecting peripheral blood vessels, cerebral ischemia, and the stroke. Ischemia can arise from several causes, such as embolisms originating from the heart, diseases affecting large blood vessels, or blockages in smaller brain vessels[2].

I/R – induced cellular damage stems from multiple mechanisms, including elevated calcium levels, disturbances in the cellular energy system, and the generation of free radicals that lead to oxidative damage within cells[3]. Furthermore, the activation of pathways dependent on reactive oxygen species (ROS) contributes to inflammation and cell death, exacerbating the damage from I/R[4]. The transient receptor potential ankyrin 1 (TRPA1) channel, a non – selective cation channel sensitive to calcium, plays a role in increasing intracellular calcium levels. TRPA1 is present among primary afferent neurons, such as those in the trigerminal and dorsal root ganglia, and is linked to the perception of pain[5]. Recent research also indicates that the presence of TRPA1 channels among non – neuronal tissues, such as lungs, heart, liver, kidney, and intestines. TRPA1 is triggered due to variety of external irritant particles and natural substances, likely allyl isothiocyanate, wintergreen oil, cinnamaldehyde, mustard oil, cannabidiol, allicin, and chemicals. It is crucial in sensing detrimental cold and internal stimuli, including products of oxidative and cytokines. Specific antagonists, including HC-03001, A-967079, and AP-18, are known to bind specifically to the TRPA1[6].

There is a known association between hypoxia, an increase in ROS, and a surge in unsaturated aldehyde like 4-hydroxy-2-nanorenal (4-HNE), which naturally activate the TRPA1 channel[7]. TRPA1 becomes active during ischemic conditions through the oxidation or modification of its cysteine residues by free radicals. The process of I/R causes an enhancement in ROS, which initiates inflammation and immune responses in affected tissues. Cells involved in the inflammatory response, such as macrophages, release various cytokines as well as chemokines, and these cells also express TRPA1[8]. While few studies highlight potential of TRPA1 as a therapeutic agent in I/R injuries, others argue that its activation may actually intensify ischemic damage. This review explores the role of TRPA1 in I/R injuries among various organs, focusing on intricate mechanisms.

TRPA in Cerebral Ischemia

Disruptions in cerebral blood circulation can swiftly cause permanent neuronal damage. The blood vessels in the brain are uniquely adapted to regulate blood flow under various conditions, thus protecting the brain from harm[9].

Inflammation induced by global cerebral ischemia / reperfusion (I/R) aggravates neuropathic pain and negatively impacts the spinothalamic tract, altering sensory neuron function. Studies have found that the absence of TRPA1 signaling in lining of cerebral blood vessels exacerbates brain interactions in models like permanent middle cerebral artery occlusion (MCAO) in mice[10]. On the other hand, pharmacological enhancement of TRPA1 has been observed to decrease these infarctions, a benefit reduced in the absence of TRPA1 channels in endothelium[11]. The anesthetic isoflurane, commonly utilized in MCAO procedures, reportedly boosts TRPA1 activity. According to Pires et al., isoflurane activates TRPA1, leading to the dilation of cerebral arteries and reducing ischemic damage[12].

In contrast, some research point out that pharmacologically activating TRPA1 might bleed to increased loss of brain tissue in ischemic strokes. A study found that cilostazol mitigated neuropathic pain in rats following global cerebral I/R caused by occlusion of bilateral carotid artery[13]. This effect was linked to the deactivation of the NLRP3 inflammasome, stimulation of the NRF2 axis, as well as promotion of neuron survival and dopamine signaling. Cilostazol was also found to suppress TRPA1 and excitotoxicity, which are increased by cerebral I/R. Neuroprotective properties of cilostazol are attributed to TRPA1 inhibition, activation of the Akt pathway, enhancement of brain – derived neurotrophic factor (BDNF), and stimulation of NRF2 axis.

Further research indicates that TRPA channels can damage myelin and contribute to white matter loss during stimulated ischemia. Agents like carvacrol and JT010, which activate TRPA1, have been found to obstruct myelination and induce myelin loss in cortical slices[14]. Conversely, the selective TRPA1 antagonist A-967079 lowered calcium levels in oligodendrocytes and increased optic nerve action potential. TRPA1 activators, such as polyhodial and AITC, were seen to reduce action potential amplitude in optic nerve, an effect negated through A-967079[15]. Blocking TRPA1 has been shown to preserve action potential during oxygen – glucose deprivation (OGD) and facilitate recovery, suggesting that TRPA1 in glial cells regulates neuronal excitability in both health and diseased states. Zhou et al. showed the vital role of TRPA1 in demyelination caused by simulated ischemia[16]. They also observed that desflurane post – treatment reduced hypoxic – ischemic brain injury in neonatal rats through diminishing TRPA1. Additionally, using HC-030031 to inhibit TRPA1 decreased hypoxic – ischemic perinatal brain damage and improved memory and learning deficits.

TRPA1 in Cardiac Ischemia

Myocardial infarctions are the prominent cause of death and health complications worldwide. Swift restoration of blood flow in the clogged coronary artery is key to limiting damage to the heart muscle, though it can sometimes intensify the injury[17]. Research led by Lu et al. demonstrated that specific TRPA1 agonists, such as ASP-7663

and optovin, are effective in reducing death of cardiac cells while reperfusion in mouse models of heart I/R injury[18]. Although, cinnamaldehyde, a different TRPA1 stimulator, had no effect on heart attack size. This study also discovered that opioid – induced reduction in infarction size is facilitated through TRPA1, as its blockade pharmacologically impaired the capacity of morphine to less infarct size. Furthermore, activating TRPA1 with optovin and ASP-7663 either prior or amidst hypoxia – reoxygenation safeguarded adult mice cardiac cells, an effect that was reversed by inhibitors AP-19 and TCS-5861518. Notably, there is an interplay between TRPA1 and the opioid system, affecting the analgesic mechanism of morphine[18].

The research also delved into the systemic consequences of natural ingredient – based topical analgesics, such as IcyHot cream containing methyl salicylate. This study by Wu et al. suggested that topical application of this cream lessened infarction size by elevating blood levels of methyl salicylate, this safeguarding rats against cardiac I/R through TRPA1[19]. This protective mechanism was inhibited by TRPA1 blockers. Moreover, exposure of cardiomyocytes to methyl salicylate during reoxygenation led to decreased cell death, and effect counteracted by TRPA1 antagonists.

Another aspect of TRPA1 involves its activation by AITC, which bolsters the survival of cardiomyocytes post – ischemic, improving their contractile ability via Akt and eNOS phosphorylation and increased calcium influx. Regarding cardiac fibroblasts, excessive TRPA1 expression significantly fostered their transformation into myofibroblasts, a change resisted by TRPA1- deficient cells[20,21]. The increase in TRPA1 expression, stimulated by TGF-β, activated the calcineurin pathway, facilitating this transformation.

Conversely, recent research indicated that manipulating TRPA1 activity, either through activation or inhibition, did not impact the size of myocardial infarction in rats[22]. Moreover, in combined of adult murine sensory neurons and cardiomyocytes culture, a slight enhancement in cardiomyocyte survival was observed under I/R conditions, linked to TRPA1[23]. In another study it was showed that TRPA1, present on membranes of cardiomyocytes, could be targeted by acrolein, a major byproduct of lipid peroxidation[24]. Apparently, they noted that harm induced by acrolein was considerably less in TRPA1-deficient mouse cardiomyocytes, with a decrease in calcium overload and hypercontraction. Moreover, the TRPA1 inhibitor HC-030031 not only alleviated infarction – related cardiac dysfunction but also reduced cell death and fibrosis while promoting angiogenesis in mice. This treatment was seen to alter various cellular pathways and expression, influencing endothelial cell activity[24].

Ustenel et al. focused on the effectiveness of iloprost and β3 adrenergic receptor agonist among channels of TRPA1 and TRPC1 in heart I/R injury in mice. The findings indicated elevated levels of oxidants and these ion channels in the I/R group, but the treatments did not significantly change the levels of TRPA1 in comparison to I/R subjects[25].

TRPA1 in Peripheral Ischamia

Peripheral artery disease (PAD) often leads to ischemia of limb, resulting in a condition known as dysesthesia, marked by pain and a numbing sensation after blood flow is restored[26]. Studies indicate that during ischemic incidents, the activation of TRPA1 by oxidants is a major factor in establishment of pain and various abnormal skin sensations, including itchiness and licking behaviors following ischemia[8].

CRPS1, a condition that frequently arises following surgery, fracture, or limb ischemia, is characterized by severe pain[27]. To investigate the pathology of PAD and its associated ischemic pain researchers often use models to long – lasting hindilimb ischemia. Klafke et al. highlighted the critical role of TRPA1 in both the acute chronic pain associated with CPIP in a rat model, a condition resulting from I/R. This model displayed an increased sensitivity to cold and touch and an upsurge in inflammatory and oxidative stress markers. Treatment with HC-030031 was found to be effective in alleviating these pain symptoms[28].

In CPIP – afflicted mice, the permanent deactivation of TRPA1 markedly reduced their sensitivity to touch and cold. Temporary relief was also achieved through the pharmacological blocking of TRPA1 using specific antagonists or α-lipoic acid. Injured nerves in mice subjected to I/R exhibited a higher concentration of macrophages and oxidative stress markers, unlike in TRPA1 – deficient mice. Deactivating TRPA1 in Schwann cells led to a decrease in macrophage activity and reduced pain sensitivity[28].

Dysesthesia, an atypical sensation often associated with neuropathy or vascular damage, can manifest following reperfusion after ischemia. Research involving temporary limb I/R in mice implicated TRPA1 in dysesthesia – like behaviors and post – ischemic licking, which were alleviated by ROS scavengers or pre – treatment with HC – 030031[29]. In models of diabetic neuropathy in mice, mechanical and cold hypersensitivity reached a peak following the induction of diabetes, with the involvement of TRPA1 being evident[30].

Research by Hiyama et al. showed that in diabetic mice, cold sensitivity was reduced through TRPA1 inhibition or removal, although mechanical hypersensitivity remained unaffected[31]. Following diabetes induction, TRPA1 activation triggered nocifensive behaviors, which were mitigated by Tadalafil, a drug that also improved blood flow.

The ligation of femoral artery in mice serves as vital for examining PAD in humans. Studies by Xing et al. revealed that blocking the femoral artery led to an increase in TRPA1 levels in DRG neurons, especially in C-fiber afferent nerves. This rise in TRPA1 was moderated by HC-03001. Additionally, TRPA1 in rat DRG neurons was found to interact with PAR2, intensifying TRPA1 – induced currents and showing increased expression after arterial occlusion[32].

In vitro experiments subjected cells expressing TRPA1 and mouse DRG neurons to hypoxia, followed by treatment with hydrogen peroxide in

order to mimic I/R damage[33]. The findings indicated that both agents activated TRPA1, with hypoxia specifically intensifying TRPA1 sensitivity in DRG neurons. Acrolein, a highly reactive neurotoxic lipid peroxidation product and a known TRPA1 activator, was associated with increased sensitivity to post – I/R spinal cord injuries. Treatment with Phenelzine lessened this hypersensitivity, diminished acrolein levels, and curbed the upregulation of TRPA1, aiding in the survival of motor neurons[34].

TRPA1 in Retinal Ischemia

Vision – impairing illnesses, such as retinal vein occlusion and glaucoma, are related to I/R in retina. This affliction is characterized by oxidative stress, neuronal depolarization, calcium influx, and inflammation[35]. Studies indicate that eliminating TRPA1 genetically or through drug intervention can protect cells of retina cells from ischemia – related injury, typically observes in rats. In studies involving mice with retinal injuries due to I/R, the absence of TRP1 or application of TRPA1 – blocking eye drops (HC-030031 and A-967079) led to decreased caspase-3 activity, a reduction in retinal cell mortality, and the maintenance of retinal thickness[36]. Treatment of α-lipoic acid demonstrated comparable benefits, pointing to role of both oxidative stress and TRPA1 in causing tissue damage. However, removing TRPV1/TRPV4 had not conferred any defensive benefits against I/R – induced injury. Corroborating these observations, a study reported increased TRPA1 levels among chick retinas post – oxygen and glucose deprivation (OGD)[29]. Although activating TRPA1 with mustard oil had not impacted the release of lactate dehydrogenase (LDH) in retinal cells during OGD, blocking TRPA1 with HC-030031 did inhibit LDH leakage in ischemic conditions. Moreover, combining mustard oil and WIN55212-2 notably escalated release of LDH. Yet, the induced cell death was averted by the use of AM251 and O-2050 or AM630[29]. Further research revealed that A-967079 enhanced action potential among optic nerves from mice. In OGD mode, a decline in amplitude of action potential was associated with a rise in calcium influx[37]. In line with this, inhibiting TRPA1 during OGD was found to preserve the amplitude of action potentials in the optic nerve.

TRPA1 in Lung Ischemia

Lung ischemia – reperfusion (I/R) injury emerges as a critical issue post – cardiac bypass or lung transplant surgeries. The intricate response of lungs to I/R is attributed to its distinct dual blood flow and oxygen supply system, differentiating it from other organs[38].

Sensory nerves within the lungs are crucial for managing respiratory balance and homeostasis. A substantial number of these nerves, particularly afferents of vagus nerve, react to stimuli. In pathological scenarios, triggering TRPA1 within these nerves can induce neurogenic inflammation. Studies involving rat models of lung I/R damage revealed elevated levels of oxidation stress indicators in the commissural nucleus of the solitary tract of brain[39]. Concurrently, a decrease in the expression of the antioxidant

transcription factor NRF2 in the brain stem was notes, alongside an upregulation of NADPH oxidase 4 (NOD4) as well as TRPA1. Blocking of NOX4 activity led to a decrease in these oxidative stress markers and mitigated the heightened expression of TRPA1 post – lung I/R damage[40]. Similarly, using pharmacological methods to inhibit inflammatory cytokines also resulted in reduced TRPA1 overexpression in the sensory nerves after lung I/R damage[41]. Corroborating these results, another study highlighted that TRPA1 and sensory proteinase – activity receptor – 2 (PAR2) experiences increased expression among lung I/R scenarios. The use of FSLLRY-NH2 to inhibit PAR2 effectively reduced the overexpression of TRPA1 through mechanisms involving signaling pathways of p38 – MAPK and c-Jun-N-terminal kinases (JNK). Furthermore, targeting receptors specific to individual proinflammatory cytokines also brought down the levels of TRPA1 and PAR2 in vagal afferent nerves of lungs[42].

Discussion

Ischemic events in important organs like heart and brain are often critical and potentially fatal. A notable link exists between ischemia/reperfusion (I/R) and a range of conditions such as ischemia of kidney and lung, myocardial infarction, disorders of the peripheral vascular system, and ischemia in the brain and retina[37]. TRP channels, notably TRPA1, are known to facilitate the passage of calcium and other cations, suggesting their role in cellular and organ damage post – I/R[8]. The involvement of TRPA1 in I/R across various organs has been documented, but the results are varied and occasionally conflicting, likely due to differences in experimental approaches, animal subjects, or the specific organs examined.

In models like hindilimb ischemia and peripheral neuropathy, which stimulate peripheral artery disease (PAD), the spontaneous pain observed during reperfusion was lessened by either genetically removing or pharmacologically inhibiting TRPA1[43]. The primary damage in cardiac ischemia occurs during reperfusion, marked by excessive oxidative stress that damages cardiac tissue. The influx of calcium through TRPA1 may exacerbate this damage. Research indicate that TRPA1 – deficient mice experienced smaller cardiac infarctions and less cellular damage[44]. Conversely, studies on animals demonstrate that TRPA1 could amplify lung ischemic damage by initiating inflammatory and oxidative stress response[45,46]. Yet, few *in vitro* researches have shown that stimulating TRPA1 actually aids in the survival of cardiac cells post – ischemia and enhances cardiac muscle contraction by activating Akt phosphorylation and increasing cardiac cell calcium levels[47,48].

In cases of brain ischemia, this condition triggers acidosis, TRPA1 activation, and a rise in intracellular calcium. Animals without TRPA1 exhibited less ischemic damage, a finding supported by the application of TRPA1 antagonists[29]. However, the potential protective role of TRPA1 in brain ischemia remains a subject of debate. For example, in cerebral ischemia models, rodents lacking TRPA1 in endothelial

cells displayed bigger infarcts, implying that natural agonist activation of the TRPA1 may offer protecting effects[49].

The progression and testing the oral antagonists of TRPA1 in trials will provide more understanding of this role of channel in ischemic diseases. Further research should aim to investigate the impact of TRPA1 on ischemia in other organs as well.

Conclusion

The current review underscores the dual nature of TRPA1, like other channels of TRP, in exhibiting either beneficial or harmful roles during ischemic events. These divergent outcomes suggest that TRPA1 serves a mandatory function within the body, triggering different responses when activated. Fundamentally, TRPA1 emerges as a promising candidate for ischemia intervention, with its impact potentially differing according to the level of activation, the location of ischemia, and the intensity and duration of the ischemic condition.

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