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Immune and inflammatory mechanism of remote ischemic conditioning: A narrative review

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Abstract:

The benefits of remote ischemic conditioning (RIC) on multiple organs have been extensively investigated. According to existing research, suppressing the immune inflammatory response is an essential mechanism of RIC. Based on the extensive effects of RIC on cardiovascular and cerebrovascular diseases, this article reviews the immune and inflammatory mechanisms of RIC and summarizes the effects of RIC on immunity and inflammation from three perspectives: (1) the mechanisms of the impact of RIC on inflammation and immunity; (2) evidence of the effects of RIC on immune and inflammatory processes in ischaemic stroke; and (3) possible future applications of this effect, especially in systemic infectious diseases such as sepsis and sepsis-associated encephalopathy. This review explores the possibility of using RIC as a treatment in more inflammation-related diseases, which will provide new ideas for the treatment of this kind of disease.

Keywords:

Inflammation, ischemic stroke, remote ischemic conditioning, sepsis

Introduction

Remote ischemic conditioning (RIC) refers to one or more cycles of a brief period of blood flow interruption to an organ or tissue remote from the target organs followed by the resumption of flow, which has been demonstrated to protect against more severe ischemic events in the target organs. RIC has many advantages, including convenience, noninvasiveness, economy, practicality, few adverse reactions, and ease of popularization. This far-reaching phenomenon was first reported by Murry and colleagues.[1] Since then, RIC has been widely studied and has shown multi-organ benefits. In cardiovascular diseases, cross-species cardiovascular protection by RIC was observed in studies of ischaemia, and this method was highly effective in preventing myocardial infarction-induced damage in animal models and humans. In cerebrovascular diseases, it has been determined that RIC could increase brain tolerance to injury caused by ischemia, reduce the risk of cerebral infarction, improve blood flow to the brain, and promote the formation of alternately routed blood vessels to the brain.[2-4] Except for local vascular diseases, recent research has expanded its application to systemic vascular diseases such as essential hypertension.[5] RIC is also effective in ischemia-reperfusion (I/R) injury after organ transplant, including kidney and liver transplants, and can improve the survival outcomes associated with systemic inflammation. However, in these conditions, the clinical effectiveness of RIC has not been fully confirmed. The mechanisms of RIC have been deeply studied in animal models and clinical trials. RIC exerts its effects through the nervous, endocrine, and immune systems. Current research revealed that inhibiting apoptosis, reducing oxidative

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stress injury and inhibiting inflammatory response is involved in this procedure.[6,7]

Immune modulation and the inflammatory response play important roles in many diseases. The regulation of immunity and inflammation has become an important therapeutic strategy.[8] At present, although a large number of studies have gradually revealed the effect of RIC on the immune system, its mechanisms have not been fully clarified, and its effect on the immune system in diseases has not been confirmed. With the prevalence of COVID-19, the increased mortality of infected people with a variety of underlying diseases, including cerebrovascular diseases, has aroused widespread concern.[9] Based on the extensive effects of RIC on cardiovascular and cerebrovascular diseases, the possibility of using RIC as an intervention in more inflammation-related diseases has been explored, which will expand new ideas for the treatment of this kind of disease, and this article reviewed papers related to immunity and inflammation since the concept of RIC was first discovered and summarized the effects of RIC on immunity and inflammation from three aspects: (1) the possible mechanisms of the effects of RIC on immunity and inflammation; (2) evidence of the effects of RIC on immune and inflammatory processes in ischaemic stroke; and (3) possible future application direction, especially in systemic infectious diseases such as sepsis.

Molecular and Cytological Mechanisms of Remote Ischemic Conditioning

Molecular regulatory mechanism

Adenosine and the A2 receptor

Adenosine is a master regulator of energy metabolism in the immune system, and the recognition of cellular stress initiates and inhibits inflammation.[10] Extracellular adenosine build-up caused by hypoxia may be a key immunoregulatory signal. The downregulation of an overactive inflammatory response is thought to be triggered by A2 receptors, which are one of the four subtypes of adenosine receptors. During the course of an immune response, extracellular A2 receptors serve as both the primary sensor of tissue damage and the catalyst for the downregulation of hyperactive immune cells. Regulation by extracellular adenosine protects normal organs from injury and/or redirects immune responses.[11] The cardioprotective effects of remote conditioning are linked to anti-inflammatory properties mediated by the subsequent activation of the cyclic adenosine monophosphate (c-AMP)/c-AMP-dependent protein kinase A (PKA)/nuclear factor kappa beta (NF-κB) axis according to Xu *et al*., who performed rhythmic compression of the forelimb in a murine model of myocardial infarction.[12] Activation of the A2 adenosine receptor by adenosine and the subsequent stimulation of the cAMP/PKA signalling pathway, which inhibits NF-κB-mediated production of inflammatory cytokines, are the mechanisms by which inflammation is downregulated. In this study, NF-B p65 phosphorylation caused by acute myocardial infarction (AMI) could be greatly reduced by remote conditioning, as shown by western blot analysis of myocardial tissues. Tumor necrosis factor-alpha (TNF-α) and Interleukin-1 (IL-1) β expression is suppressed by a decrease in NF-κB activation. After three days of remote conditioning, serum and heart adenosine levels were measured. The results suggested that serum adenosine levels were increased, and remote conditioning increased adenosine levels in the heart. Additionally, the researchers discovered that remote training significantly enhanced the mRNA levels of A2a and A2b receptors in the hearts of AMI rats, which demonstrated that remote conditioning induced A2 receptor expression in the heart. In contrast, remote conditioning plus the selective A2A antagonist SCH 58261 or the selective A2b antagonist MRS 1754 induced a reversible anti-infarct effect compared to that in AMI rats treated with remote conditioning alone.

Cytokines and signalling pathways

ERK/HMGB1 pathway

Local immune cells identify damage-associated molecular patterns (DAMPs) and necrotic tissue in the infarct area after reperfusion.[13] High mobility group protein B1 (HMGB1), extracellular DNA, and histones are examples of "alarmin" molecules that bind to DAMPs and cause the NF-B pathway to mediate the secretion of proinflammatory cytokines.[14] To control innate and adaptive immunity, the highly conserved DNA-binding protein HMGB1 is passively released by dead cells or actively secreted into the extracellular environment by inflammatory cells.[15] Receptors for advanced glycation end products (RAGE), toll-like receptor (TLR) 2, and TLR4 are known HMGB1 receptors.[16] According to previous studies, RAGE is the immunoglobulin superfamily's earliest discovered HMGB1 receptor.[17,18] RAGE can bind to its ligands and activate several protein kinases, including Janus kinase/STATs, Rac/cell division control protein homologue, and MAPKs, which can then activate the NF-B signalling cascade.[19] ERK, which is one of the most characteristic members of the MAPK family, controls a variety of cellular processes and functions, including cell metabolism, viability, inflammation, necrosis, and apoptosis.

Studies have demonstrated the effects of RIC on the ERK/HMGB1 pathway. According to Xu *et al*.,[12] by inhibiting the ERK pathway, remote ischemic postconditioning (RIPOC) was able to protect mice suffering from intestinal I/R damage. In this study, a mouse model of intestinal I/R injury was treated with

RIPOC and/or an ERK inhibitor (CC-90003). The research showed that RIPOC could reduce the histopathological features of the intestinal mucosa in intestinal I/R-damaged mice through the ERK pathway. In addition, RIPOC significantly decreased the expression of HMGB1 and RAGE in mouse models ($P < 0.05$), and these decreases were linked to ERK pathway inhibition. Furthermore, in intestinal I/R damage mouse models, RIPOC was shown to inhibit the NF-κB (p65)/nlr family pyrin domain containing 3 (NLRP3) inflammatory pathway via the ERK pathway. Another study used a mouse model of hepatic I/R injury and showed that RIC significantly decreased the levels of intrinsic liver enzymes, IL-6, and TNF-α. This effect was mediated by the HMGB1/TLR4/NF-B pathway.[20] These studies showed that RIC could inhibit the ERK/HMGB1/RAGE/NLRP3 pathway or the HMGB1/TLR/NF-κB pathway, which could reduce the levels of proinflammatory cytokines and thus inhibit inflammatory responses.

Interleukins and tumour necrosis factor

Interleukins and tumour necrosis factor act as messengers between cells and tissues throughout the inflammatory process. Proinflammatory and anti-inflammatory cytokines work in opposition to each other to keep the immune response in balance. The proinflammatory cytokines IL-1, IL-1, IL-6, TNF, IL-8, and IL-18 are released in response to myocardial infarction. A combination of damaged cardiomyocytes, macrophages, and an activated endothelium releases these cytokines.[21,22] The main goal of cytokine production is to stimulate and attract immune cells to the site of inflammation, allowing for the removal of damaged products via phagocytosis.[23] TLR4 signalling pathways, the activation of NF-κB in circulating macrophages, and reactive oxygen species (ROS), which interact with IL-6, cause cytokine production in the infarcted heart. The subsequent IL-1 β release induces the production of more proinflammatory mediators.[24,25] According to previous studies, RIC can reduce the production of proinflammatory cytokines. Kim *et al*.[26] investigated the effects of remote ischaemia preconditioning (RIPC) and RIPOC on serum cytokines in LPS-induced septic mice. The results demonstrated that TNFα-, IL-1 and IL-6 were significantly increased in mice that had just received LPS injections; however, remote ischaemia training prevented these increases. To identify molecular mechanisms in kidney tissue that could be linked to RIC, Kessler *et al*. used an integrative omics technique combining transcriptomics, proteomics, and phosphoproteomics. The researchers carried out a randomized clinical study (CONTEXT) to compare the effects of RIC to non-RIC controls in human kidney transplants. The researchers also investigated whether RIC caused molecular changes by carefully analysing recipient plasma and kidney tissue samples in CONTEXT using high-resolution tandem mass spectrometry (MS). Despite the fact that RIC did not improve the clinical outcomes of transplants, the researchers did notice the accumulation of proteins derived from muscles and altered amino acid metabolism in kidney tissue proteomes, which may have been caused by RIC but was not seen in plasma.

The anti-inflammatory reparative phase promotes scar formation and wound healing. Additionally, cytokines are important for tissue healing. Following I/R, IL-6, IL-10, and TGF-β are associated with reducing the proinflammatory response and directing the immune system towards healing and resolution.[25,27] Billah *et al*. found that RIC was associated with an increase in IL-6 via the zinc finger transcription factor early growth response-1 (EGR-1),[28] which is upstream of many apoptotic pathways and is related to IL-6 mRNA expression. The particular procedure will be explained in the "Transcription Factor" section. In the early stages of hepatic IR, IL-10 orchestrates the production of proinflammatory cytokines and chemokines. After 15 min of complete hepatic ischaemia and 24 h after reperfusion, genetically obese mice that were pretreated with exogenous IL-10 had a much higher chance of surviving.[29] Prior to myocardial I/R, RIC increased the levels of the protective cytokine IL-10,[30] which governs the amplitude of the cytokine response.[31,32] Signal transducer and activator of transcription 5 (STAT5) is responsible for this increase in IL-10 *in vivo*.[30] STAT5 is linked to the survivor activating factor enhancement pathway and acts downstream of JAK (Janus Kinase) in human myocardial injury.[33] Similarly, previous literature has discussed the protective effects of IL-10, which can limit I/R injury via STAT3.[34-36] RIC could also increase IL-10 during liver transplantation. The hepatoprotective effects of RIC were examined by Czigany *et al*. in a rat model of arterialized orthotopic liver transplantation (OLT). When compared to the control 1-h group, the RIC 11-h and RIC twenty-one-hour groups had significantly higher levels of IL-10.[37]

HO-1

HO-1 is a stress protein (Hsp32) and a key enzyme associated with haem catabolism.[37] Through its immunomodulatory, antiapoptotic, and vasoactive qualities, HO-1 and byproducts of hemcatabolism (such as carbon monoxide and biliverdin) protect against transplanted IR harm.[38] In addition to its key anti-ischemic regulatory role, the antirejection features of HO-1 have also been demonstrated in liver transplantation. Wang *et al*. conducted a thorough experimental investigation that demonstrated the powerful effects of RIC on the induction of HO-1 in a murine warm IR injury model. The scientists concluded that HO-1 may play a crucial role in autophagy and apoptosis by activating signal kinase pathways to promote autophagy, consuming

damaged mitochondria to suppress apoptosis, and ultimately protecting hepatic cells against IR injury.[39] RIC was also found to substantially increase HO-1 levels in an LPS-induced sepsis mouse model.[26]

Receptors

Toll-like receptor 4

The class of protein molecules known as TLRs is crucial for nonspecific immunity (innate immunity) and serves as a link between nonspecific and specific immunity. TLRs are single transmembrane noncatalytic proteins that may identify the compounds from bacteria with conserved structures. TLRs can identify bacteria that have penetrated the body's physical defences, such as the skin and mucosa, and can then induce an immune cell response. Microglia expresses the high levels of TLR4 on their cell surface during I/R damage following stroke, which aids in the initiation of the innate immune response. Although excessive activation of these receptors worsens the inflammatory condition, moderate activation during the brief ischemic conditioned reflex may offer neuroprotection by preparing the brain for severe and prolonged ischemia. According to Pradillo *et al*.,[40] RIC can cause immunological tolerance, which prevents TLR4 expression after middle cerebral artery occlusion (MCAO) and shrinks the infarct size. TLR4 is essential for RIC to activate innate immunity and promote ischemia tolerance, as genetic deletion of the TLR4 receptor eliminates the protective effects of RIC. RIC increases TNF-α, inducible nitric oxide synthase (INOS), and NF-κB levels. Downregulation of the transcription factor cyclooxygenase-2 (COX-2) and p65 subunit protein levels was observed. The ischaemia tolerance induced by RIC is thoughts to be mediated by these molecular proteins. Similarly, TLR4-deficient animals had the opposite outcomes. RIC-induced neuroprotection was mediated by the TLR4 signalling pathway, which first activated the transcription factor NF-κB and subsequently upregulated TNF-α, INOS, and COX-2 levels.

According to previous studies, RIC works better when paired with other therapies to lower TLR4 expression. Cheng *et al*.[41] examined whether the combination of RIC and astragaloside IV could enhance cardioprotection against AMI-induced heart failure compared to monotherapies. To determine the expression of TLR4 and its downstream protein NF-κB in rat cardiac tissues, western blotting was used. In comparison to those in the model group and individual treatment groups, combination therapy significantly reduced the levels of TLR4 and NF-κB.

Chemokines and their receptors

Small heparin-binding proteins called chemokines guide the migration of circulating leukocytes to injury or inflammatory sites. The primary purpose of chemokines is to encourage the cells to migrate in a specific direction. The chemokine-attracted cells move toward the chemokine source in response to the increasing chemokine concentration. Monocyte chemoattractant protein 1 (MCP-1), which is often known as "chemokine ligand CCL2," is the most well studied CC chemokine. It is a strong agonist for basophils, memory T cells, dendritic cells, and monocytes. Multiple effects of CCL2 have been observed (chemokine for monocytes, contributing to apoptosis and biliary fibrosis). The only recognized receptor for CCL2 and CCL13 is CCR2.[42] The expression of the CCR2 gene is inhibited by remote ischaemic preconditioning (RIpreC).[43] This finding is consistent with the considerable reduction in closely adherent leukocytes and leukocyte accumulation at the site of inflammation in CCR2-defective animals, which suggests that the regulatory effect of RIC on CCR2 may reduce leukocyte adherence.[44] There was no significant difference between the RIC groups and the control group, which is interesting because Czigany *et al*.[37] found a significant increase in serum MCP-1 levels in a rat model of arterialized OLT on the first postoperative day and slightly lower levels after RIC. These findings suggest that RIC may suppress CCR2 gene expression but not lower CCL2 synthesis to exert its anti-inflammatory effects.

The family of chemokines known as macrophage inflammatory protein-1 includes CCL15. The genetic sequence of CCL15 is comparable to that of CCL5, which is known to be produced and released in response to normal T-cell activation. The primary chemoattractant function of CCL15 is mediated by the CCR1 receptor. In isolated human blood monocytes and eosinophils, CCL15 causes a brief increase in intracellular calcium.[45] He *et al*.[46] discovered that in a mouse model of doxorubicin-induced cardiotoxicity, RIC could dramatically reduce the increase in CCL15.

Complement

RIC-mediated neuroprotection may also be influenced by early alterations in the complement response. Song *et al*.[47] investigated the peripheral blood proteome responses in adult monkeys with stroke following short-term and long-term RIC. To perform a proteome analysis using MS, plasma samples from two adult rhesus monkeys that had autologous blood clots that caused middle cerebral artery (MCA) blockage underwent RIC twice per week for five consecutive weeks. Complement C3, C4b-binding protein beta chain, and complement C1 subcomponent were increased. This finding suggests that the classical pathway of the complement system was activated in response to RIC. One week after RIC, C-type lectin was highly abundant and involved in the innate and adaptive antimicrobial immune responses.

Gene transcripts related to inflammation regulation

Transcription factors

Antioxidative response elements (AREs) in the promoter regions of antioxidative genes can be bound by Nrf2 to start their transcription. In the healthy state, Nrf2 binds to the protein Kelch-like ECH-associated protein 1 (keap1), which mediates Nrf2 ubiquitination and eventual degradation in the cytosol. Cysteine residues in Keap1 allow it to function as a redox sensor under oxidative stress conditions, identifying changes in the cellular redox state. Keap1 is rendered inactive by the oxidation of cysteine residues (C151, C273, C288, C613), which also stabilizes and translocates Nrf2 to the nucleus. Nrf2 forms a heterodimer in the nucleus with the protein MAF, binds AREs and activates antioxidant and detoxifying enzymes. According to Guan *et al*., RIC plasma suppresses neutrophil activity by upregulating the transcription factor Nrf2 and downstream antioxidative genes, resulting in a reduction in the formation of ROS. In an experiment, zebrafish were microinjected with RIC-exposed mouse plasma. RIC plasma reduced the amount of ROS produced in response to tail damage. PCR array results revealed that RIC plasma therapy increased antioxidative-related genes such as hsp70, hmox1a, and nqo1 while decreasing H2O2 production.[48]

Because EGR-1 overexpression enhances the expression of inflammatory and prothrombotic pathways, it is crucial to the biological response to I/R. The relationship and/or function of Egr-1 with the molecular systems associated with the cardioprotective effects of RIC were established by Billah *et al*.[28] *In vitro*, H9C2 cells and a rat model of cardiac I/R damage were examined. The researchers used DNAzyme (ED5) to silence Egr-1 *in vivo* and *in vitro*. Following ED5 pretreatment before RIPC *in vivo*, there was a substantial increase in infarct size compared to that in the controls after the procedure. There was also a decrease in plasma IL-6 levels, the downregulation of the cardioprotective JAK-STAT pathway, and an increase in cardiac endothelial dysfunction. Cells that received preconditioned media from the DNAzyme-treated donor cells exhibited an increase in apoptosis and the loss of mitochondrial membrane potential as a result of ED5 administration, which eliminated IL-6 mRNA expression in H9C2 cells subjected to RIPC *in vitro*. According to the findings of this study, Egr-1 acts as a master regulator of distant preconditioning, exerting a protective effect against myocardial I/R injury through IL-6-dependent JAK-STAT signalling.

Changes in gene expression

Shimizu *et al*.[49] published the first investigation on the expression of the human leukocyte genome after RIC. The researchers discovered that the expression of proinflammatory genes in leukocytes was dramatically downregulated by the RIC in healthy individuals. TLR4 signal transduction and proinflammatory cytokine release TNF-α Genes for leukocyte chemotaxis and extravasation (pi3kca), leukocyte adhesion, and exocrine and secretory granule release are suppressed genes and are responsible for the Inflammatory response (SNAP-23). The functional response of neutrophils, particularly the notable decline in neutrophil adhesion and phagocytosis, was highly linked with similar alterations in human leukocyte gene expression in a study conducted by the same research team in 2010.[50]

Numerous genes may continue to respond to RIC at the transcriptional level. O'Brien *et al*.[51] discovered through transcriptomics that the transcript levels of heat shock protein beta-1 (HSPB1), monocarboxylate transporter 4 (SLC16A3), C-X-C motif chemokine (CXCL13), C-C motif chemokine 2 (CCL2), interleukin-1 receptor type 2, interleukin-1 beta (IL1B), leukotriene B4 receptor 1 (LTB4R), Na(+)/H(+) exchange regulatory cofactor NHE-RF4 (PDZD3), and Ras-like protein family member 10A (RASL10A) were significantly downregulated following RIC. The most noticeable changes in transcript levels were in SLC16A3, IL1B, LTB4R, RASL10A, and CXCL13; however, these changes were not observed at the protein level.

Cell differentiation, transport and restoration

RIC directs the differentiation and transport of leukocytes, including monocytes, T-cells, and B-cells. Monocytes are a significant source of inflammatory mediators and important participants in the pathogenesis of stroke. Two different subsets of circulating murine monocytes have been identified; Ly-6C and C-C chemokine receptor type 2 (CCR2+) are both highly expressed by classic monocytes. Ly-6Chigh/CCR2+ monocytes are traditionally activated M1 macrophages that are attracted into inflamed tissue in a CCR2-dependent manner. This subset promotes inflammation by producing cytotoxic and inflammatory substances. Nonclassical monocytes do not express CCR2 and only moderately express Ly-6C. This anti-inflammatory subgroup keeps performs immune surveillance in the blood vessel lumen, regulates vascular homeostasis, and promotes tissue remodelling.[52,53] In stroke models, the Ly-6Chigh subgroup reduces ischemic damage by supporting microvasculature stability and eliminating debris. According to Yang *et al*.,[54] mice that were given poststroke RIC had circulating monocytes that changed to a proinflammatory CCR2+ subtype, had less acute brain damage and oedema, and had better motor/gait function in chronic stroke. Regardless of the severity of the damage, behavioral improvements were observed. The magnitude of the RIC-induced proinflammatory monocyte shift depended on the severity of the injury. RIC significantly reduced the number of Ly-6Clow

monocytes in the blood, but the Ly-6Chigh subset did not increase, nor did the blood monocyte population shift towards a proinflammatory subset. Ly-6C high monocytes were being delivered to the injured brain because an RIC-induced monocyte shift was not present in the blood of stroke animals. A change in the blood prior to their trafficking to the brain was suggested by the RIC-induced proinflammatory shift that only affected the invading CD45-high population. Using a rat model of noninvasive RIC, Liu *et al*.[55] evaluated the effect of RIC on immune cell and cytokine profiles before and after transitory MCAO. Flow cytometry showed that RIPC reversed the post-MCAO decrease in CD3+ CD8+ T-cells and eliminated the decline in CD3+/CD161a+ NKT cells in the blood. In addition, RIC significantly increased the percentage of B-cells in peripheral blood, restoring the poststroke decline in the B-cell population. Moreover, RIC significantly increased the proportion of resident CD43+/CD172a+ noninflammatory monocytes but had no effect on the proportion of CD43/CD172a+ inflammatory monocytes.

Leukocyte synaptosome-associated protein (SNAP-23), which mediates mast cell and neutrophil exocytosis, is three times lower in individuals with RIC. The fusion of particles in these cells is inhibited by the lack of SNAP-23 because it prevents the formation of ternary complexes with other SNARE proteins. Since it is well known that neutrophils primarily cause inflammation by secreting particular cytoplasmic particles containing cytotoxic agents and proteolytic enzymes, the downregulation of the SNAP-23 gene caused by RIC may partially explain how this defence works. Additionally, the decline in neutrophil chemotaxis following RIC may be due to a decrease in platelet endothelial cell (EC) adhesion molecule (pecam1 or CD31) gene expression.[49] The blood-brain barrier is thought to be stabilized and maintained by Pecam1, which is typically expressed on specific ECs, platelets, neutrophils, monocytes, and leukocytes. Pecam1 mediates passage through the vascular wall in the paracellular urinary tract during neuroinflammation, but when this protein is blocked, leukocyte migration ceases.[56] A reduction in pecam1 mRNA expression in the RIpreC group compared to the control group may prevent neutrophils from migrating into the brain.

According to previous reports, RIC affects cells in a time-dependent manner. Doeppner *et al*.[57] investigated the best time to apply RIC and its underlying mechanisms for up to three months using a model of noninvasive RIC of the hind limbs following cerebral ischaemia in male C57BL6 mice. Three distinct paradigms were used on mice subjected to RIC; the first cycle began 12 h, 24 h, or 5 days following the induction of stroke. The results demonstrated that although long-term brain injury was greatly decreased, a long delay in RIC did not result in a reduction in infarct volume on day seven when it was first applied on day five. When compared to ischaemic controls and the early phase RIC, delayed RIC on day five increased the number of B-lymphocytes and T-lymphocytes by 112.7% and 50.7%, respectively.

Remote Ischaemic Conditioning in Acute Ischaemic Stroke: Evidence of Immune Responses and Inflammation

Acute ischaemic stroke (AIS) affects millions of people annually worldwide.[58] The immune and inflammatory response is one of the most important aspects of AIS.[59] Currently, an increasing number of studies have shown that RIC can reduce infarction volume and improve long-term outcomes through the immune and inflammatory pathways.[60]

Immune and inflammatory processes in acute ischaemic stroke

The immune response in AIS is initiated by the release of DAMPs from injured cells. DAMPs are subsequently detected by immune cells bearing corresponding pattern recognition receptors, which mediate the activation of intracellular signalling pathways. Within minutes following injury, microglia and astrocytes are activated, undergo morphological changes, secrete cytokines and chemokines and recruit peripheral immune cells.[61-63] Activation of ECs in the central nervous system (CNS), platelet dysregulation and invasion of peripheral myeloid cells and lymphocytes drive the progression of inflammation, contributing to damage to the brain parenchyma and vasculature.[64] Breakdown of the blood–brain barrier takes place early after stroke and facilitates the infiltration of peripheral leucocytes to the injured brain.[65] Moreover, leucocytes further exacerbate blood–brain barrier disruption by releasing proinflammatory cytokines, ROS, and matrix metalloproteinases.

Experimental evidence

A number of studies have confirmed the effect of RIC on the immune and inflammatory processes of AIS. According to these studies, mechanisms include preventing TLR4 expression, modifying complement and reducing proinflammatory leucocytes and neutrophil chemotaxis. It is currently thought that these effects of RIC are achieved through extracellular vesicles (EVs) circulating in the bloodstream that can transmit their contents into recipient cells. EVs can be divided into different subtypes according to their sources, such as platelet-derived microvesicles (PMVs), endothelial-derived microvesicles (EMVs) and myoblast-derived extracellular vesicles. PMVs may have an immunomodulatory effect on inflammation.[66] PMVs primarily cause neutrophils

and monocytes to release inflammatory mediators such as IL-1, TNF-, MCP-1, and MMP-9.[67] Notably, PMVs can boost the immune response by promoting leukocyte-endothelial interactions[68] via PMV uptake by activated neutrophils (polymorphonuclear cells) and ECs. A recent study examined the makeup and circulatory consequences of EMVs during inflammation. The increased protein levels of c-Src kinase within the isolated EMVs from mice generated stronger adhesion and contact between neutrophils and ECs, as shown by the increased expression of adhesion molecules (ICAM-1 and VCAM-1) and integrins (CD11b) on the endothelium and neutrophils, respectively.[69]

In a rat model of focal brain injury and CNS inflammation (induced by IL-1 microinjection into the striatal region), Couch *et al*. showed that the number of circulating CD31-positive EVs derived from ECs) significantly increased in the acute phase of brain injury compared to age-matched controls.[70] Following stroke, proinflammatory proteins were increased in circulating EVs in the bloodstream, which, according to proteomic analysis, could activate peripheral immune cells and cause an inflammatory response. To pinpoint the pathway by which the protective messages of RIC travel from the remote site to the target organ, numerous investigations have been carried out. EVs could potentially carry this signal. Skeletal muscle cells may be candidates for the release of EV as a warning signal during RIC. A recent study showed that RIC could improve the function of EVs produced by skeletal muscle cells, which can act as signals to reduce inflammation.[71]

Human studies

The remote application of ischaemic conditioning provides a safe, noninvasive and clinically applicable method for treating AIS. This method often involves intermittent cycles of inflation and deflation of a blood pressure cuff around the upper arm in humans. At present, some clinical studies have proven its efficacy in AIS. In people with narrowing of the arteries in the brain, RIC may reduce the risk of recurrent stroke. In people being treated with stenting (the insertion of a metal or plastic tube) for narrowed arteries in the neck, RIC may reduce the size of new brain injuries caused by reduced blood flow. However, its effect on clinical outcomes (stroke and death) is unclear.[72-74] Among people with acute ischaemic stroke (where it had only been several hours from symptom onset), the RICAMIS randomized clinical trial was published recently and reported that among adults with acute moderate ischaemic stroke, treatment with RIC significantly increased the likelihood of excellent neurologic function at 90 days compared with typical care.[75] However, there have been limited numbers of human studies that directly focused on the effect of RIC on the immune and inflammatory processes of AIS. In a study assessing the efficacy of RIC in the prevention of stroke-associated pneumonia in patients with AIS, Zhang *et al*. reported that IL-6 and IL-1 β levels in the RIC group were lower than those in the control group in AIS patients on day 5 after admission.[76]

Based on these experiments and clinical studies, we have reason to believe that RIC plays a key role in the immune and inflammatory processes in AIS. Future studies will continue to explore the effects of RIC in the immune and inflammatory pathway of AIS, especially clinical studies, to discover more mechanisms of the effect of RIC on AIS and find more reliable biomarkers.

Possible Future Application Directions: Sepsis and Sepsis-Associated Encephalopathy

A dysregulated host response to infection results in sepsis, which is characterized as life-threatening organ failure. Sepsis is a pathophysiological disorder that can be fatal and is characterized by a systemic inflammatory response to infection. Inflammatory cytokines such as TNF-α, IL-1 β, and IL-6 are secreted by inflammatory cells and cause septic cardiomyopathy, which is a temporary or deadly cardiac failure.[77] The DAMP HMGB1 has been linked to septic cardiomyopathy and is known as a late modulator of lethality in sepsis. Myocardial dysfunction caused by sepsis reduces blood flow to systemic organs, boosts the generation of DAMPs, hastens systemic organ inflammation, and ultimately results in MOF. The frequency of multiple organ failure (MOF) and mortality in sepsis may therefore be reduced by therapeutic interventions that decrease sepsis-induced inflammatory cytokines and subsequent myocardial dysfunction. LPS alone affects Toll-like receptors, pattern recognition receptors, and intracellular inflammatory signalling, including ERK and JNK, at the cellular level.[78] TNF-α, IL-1 β, IL-6, and HMGB1 are known to act on Toll-like receptors to stimulate these inflammatory signalling pathways.[79,80] The findings of the present investigations demonstrated that LPS treatment stimulated ERK and JNK and that RIC drastically inhibited ERK and JNK activation and decreased the levels of TNF-α, IL-1 β, IL-6, and HMGB1.

Animal studies

The beneficial benefits of RIC on sepsis have been supported by some animal studies. Intestinal HO-1 expression in the endotoxic shock model was modulated by intestinal ischemia preconditioning to prevent inflammatory reactions.[81] Additionally, Wen *et al*.[82] demonstrated that chemical inducers that upregulate HO-1 protected against LPS-induced acute hepatic damage. Kim *et al*.[26] examined the possibility that RpostC

could protect against systemic inflammation induced by LPS. Mice that received both LPS injections and remote ischaemia conditioning had considerably higher survival rates within 120 h than mice that received only LPS injections. In the LPS-only group, TNF-α, IL-1 β, and IL-6 levels were increased noticeably; however, distant ischaemia training prevented these increases. Compared to mice that received LPS injections alone, mice treated with RpostC also exhibited much higher levels of IL-10. In comparison to mice that received LPS injections alone, those that also received distant ischaemic conditioning had much lower NF-κB activation and significantly greater levels of HO-1. In comparison to that in mice that received only LPS injections, neutrophil infiltration was considerably reduced in the LPS-injected and distant ischemic-conditioned mice. In an ovine model of septic shock, Orbegozo Cortes *et al*.[83] assessed the effects of ischemia conditioning on the microcirculation, organ function, and survival time. The conditioned group had a higher cardiac index and oxygen delivery at 8 h after randomization, higher mixed venous oxygen saturation and lower lactate levels at 16 h, and a higher mean arterial pressure and lower lactate levels at 20 h. Oliguria, hypotension, and death occurred later in the conditioned group than in the control group at 6 h after randomization due to improved microcirculatory variable preservation in the conditioned group. The median proportion of perfused vessels (PPV) was 91 (89-93)% versus 89 (86–90)% (P = 0.024), and there was less heterogeneity. To investigate the changes in inflammatory biochemical profiles and identify the impact of RIC on survival in a sepsis animal model, Joseph *et al*.[84] demonstrated that overall survival was higher in the experimental group than in the sham group, with the 2-hour post-RIC group having the highest survival rate.

In the 2-h post-RIC group, the hazard ratio for improved survival 5 days following LPS was 0.3 according to the Kaplan-Meier analysis. Serum levels of IFN-g, IL-10, IL-1b, and TNFa in the 114 RIC group peaked 2 h after LPS administration and then markedly decreased over the course of 24 h in comparison to the baseline. In a mouse model of LPS-induced sepsis, Honda *et al*.[85] assessed the impact of RIC on septic cardiomyopathy and related multiorgan failure. In this study, RIC with intact left ventricular systolic function reduced the LPS-induced decline in cardiac output. RIC dramatically abrogated the LPS-induced increase in TNF-α, IL-1 β, IL-6, and HMGB1. Along with decreasing ERK and JNK phosphorylation in heart, liver, and renal tissue, RIC also reduced the increases in plasma cardiac troponin I, aspartate transaminase, alanine transaminase, blood urea nitrogen, and creatinine. RIC greatly increased the rate of survival.

Human studies

A prospective single-arm trial was conducted by Kiudulaite *et al*.[86] was performed to determine whether RIC could enhance sublingual microcirculation in septic patients. Within 24 h of intensive care unit (ICU) admission, patients with sepsis or septic shock were treated with RIC. The procedure involved inflating a brachial cuff to 200 mmHg for 5 min and then deflating it to 0 mmHg for an additional 5 min three times. The process took 30 min. At the time of study enrolment, RIC was performed again at 12 and 24 h. Different time points were used to assess sublingual microcirculation, the microvascular flow index (MFI), and the PPV among tiny vessels. According to the results, the first time RIC was administered, it significantly improved MFI and PPV (P = 0.003 and 0.026, respectively); however, the second time RIC was used, it had no significant impact.

A multicentre randomized controlled trial performed by Cour *et al*.,[87] which has no results reported yet, plans to involve 180 patients admitted to an intensive care unit with documented or suspected infection and lactatemia >2 mmol/L who were treated with norepinephrine for <12 h. At the time of study inclusion, RIC will be performed again at 12 and 24 h. The mean daily SOFA score up to Day 4 following inclusion serves as the main endpoint. The requirement for organ support, hospital duration of stay, and 90-day mortality are the examples of secondary outcomes. This investigation will examine the efficacy of RIC in sepsis.

Future direction: Sepsis-associated encephalopathy

Systemic inflammation induced by sepsis can result in acute cerebral dysfunction known as sepsis-associated encephalopathy (SAE).[88] In the intensive care unit, SAE occurs in approximately 50% of sepsis patients, and some survivors continue to have cognitive problems years after their sepsis first appears. Neuroinflammation, changes in neuronal synapses, and neurovascular abnormalities are some of the factors associated with SAE. Blood-borne cytokines, cytokine receptors, and the pyrin domain-containing protein 3 (NLRP3) inflammasome are all involved in this process, which appears to have much in common with the targets of the anti-inflammatory mechanism of RIC. The impact of RIC on SAE might be a new field for research to address this severe complication of sepsis based on findings in animal models and biomarkers of SAE in animal models and humans. A majority (59%) of patients who were hospitalized with severe COVID-19 were reported to have viral sepsis caused by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2). In the ICU, more than half (55%) of patients with severe COVID-19 had delirium, which is a symptom of bacterial sepsis. However, individuals who survived COVID-19,

particularly those who experienced encephalopathy or were hospitalized, exhibited an enhanced risk of dementia throughout the subsequent six months. If RIC is shown to be effective in treating or preventing SAE, it may also be useful for SARS-CoV-2-associated encephalopathies.

Author contributions

YX wrote the first draft of the manuscript; YW contributed to manuscript revision; XJ takes full responsibility for the data, the analyses and interpretation, and the conduct of the research. All authors read and approved the submitted version.

Ethical approval and patient content

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Conflicts of interest

Dr. Xunming Ji is the Editor-in-Chief of Brain Circulation. The article was subject to the journal's standard procedures, with peer review handled independently of this Editor and their research groups.

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