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Perspectives on effect of spleen in ischemic stroke

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Abstract:

Despite decades of research, stroke therapies are limited to recanalization therapies that can only be used on <10% of stroke patients; the vast majority of stroke patients cannot be treated by these methods. Even if recanalization is successful, the outcome is often poor due to subsequent reperfusion injury. A major damage mechanism operating in stroke is inflammatory injury due to excessive pro-inflammatory cascades. Many studies have shown that, after stroke, splenic inflammatory cells, including neutrophils, monocytes/macrophages, and lymphocytes, are released and infiltrate the brain, heightening brain inflammation, and exacerbating ischemia/reperfusion injury. Clinical studies have observed spleen contraction in acute stroke patients where functional outcome improved with the gradual recovery of spleen volume. These observations are supported by stroke animal studies that have used splenectomy- or radiation-induced inhibition of spleen function to show spleen volume decrease during the acute phase of middle cerebral artery occlusion, and transfer of splenocytes to stroke-injured brain areas. Thus, activation and release of splenic cells are upstream of excessive brain inflammation in stroke. The development of reversible means of regulating splenic activity offers a therapeutic target and potential clinical treatment for decreasing brain inflammation and improving stroke outcomes.

Keywords:

Inflammation, ischemia-reperfusion injury, splenic cells, splenectomy

Introduction

Basic research has improved our understanding of the mechanisms underlying ischemic brain damage, but the translation of this knowledge into clinically effective neuroprotective strategies has remained beyond reach. Systemic thrombolysis, *in situ* thrombolysis, and *in situ* clot retrieval are clinically effective brain reperfusion strategies. However, at present, the vast majority of stroke patients do not have favorable outcomes. Although recanalization successfully achieves reperfusion in many cases, the outcome is often poor due to consequent reperfusion injury. There are many facets of reperfusion injury. Here, we focus on a

major aspect of ischemia/reperfusion (I/R) damage: pro-inflammatory cascades. These are triggered by blood-borne immune cells during reperfusion and serve as a key factor impeding recanalization.^[1] Inflammation presents a roadblock to successful neuroprotection.^[2] Mitigating inflammation may lengthen the therapeutic window for administering neuroprotectants, leading to better brain preservation and improved outcome following I/R injury.

Many studies have confirmed that, after stroke, splenic inflammatory cells, including neutrophils, monocytes/macrophages, and lymphocytes, are released and infiltrated into brain tissue to act as pro-inflammatory agents and worsen brain I/R injury.^[3] Clinical studies have observed spleen contraction in patients early after stroke, with improved function correlating with the recovery of spleen volume.^[4] Animal studies observed in the acute phase of middle cerebral

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artery occlusion (MCAO), the volume of spleen was decreased, and splenocytes transferred from the spleen to cerebral ischemic areas.^[5] Activation and release of splenic cells is thus a key proximal step inducing brain inflammation after I/R injury. Therefore, control of splenic activity after stroke may serve as a therapeutic target to decrease brain inflammatory injury and improve patient outcomes after stroke. The permanent removal of spleen, including preischemia (2 weeks before stroke) and acute splenectomy, has been widely used for spleen suppression and neuroprotection in animal studies. Whether this neuroprotection was due to reduced inflammation remains to be determined. Any clinically practical approach would reversibly suppress spleen inflammatory reactions during the early stroke to mitigate acute brain inflammation.

The brain controls humoral immune responses amenable to modulation.^[6] After ischemic stroke, the brain-spleen axis activates the spleen,^[7-9] where the inflammatory response is not only triggered by cerebral ischemia but also aggravates cerebral I/R injury. Numerous studies have confirmed that the spleen is a reservoir of immune cells.^[10] During acute ischemic stroke, multiple signals are produced by the injured brain that initiates brain-spleen communication. The spleen, in turn, releases a variety of immune cells which act on the brain, leading to neuroinflammation. The spleen is significantly reduced in volume after stroke. Interest in the spleen as a potential therapeutic target for immunomodulation has increased. If effective, this would be an important breakthrough in stroke treatment. Previous animal studies showed that total splenectomy- or radiation-based suppression of spleen blocked spleen activity and decreased brain inflammation after I/R.^[11,12] However, splenectomy and other experimental methods that permanently suppress spleen are not clinically practical. We will now discuss the mechanisms of spleen-induced neuroinflammation. These mechanisms suggest potential means of reversible modulation of this activity and hence potential clinical applications.

The Key Role of Spleen in Ischemia/Reperfusion Injury

Activation and release of inflammatory cells from the spleen after stroke are an important sources of the central immune response. Studies have shown that the activation and central infiltration of splenic macrophages by monocyte chemoattractant protein-1 (MCP-1) and its receptor C-C chemokine receptor 2 (CCR2) are the main sources of macrophages in early cerebral ischemia.^[10] Reduced expression of Ly6C^{high} and CCR2 in spleen-derived pro-inflammatory macrophages was associated with reduced infarct volume.^[13] Another study using labeled immune cells combined with

fmMRI demonstrated infiltration of macrophages (35%) and neutrophil cells (11%) 24 h after MCAO.^[14] Interleukin (IL), tumor necrosis factor-alpha (TNF- α), and MCP-1 are classic inflammatory factors released by macrophages, which further regulate the release and migration of inflammatory cells, such as neutrophils, natural killer cells, and lymphocytes.^[15] The infiltration of inflammatory cells and cytokines, which were increased in the injured cerebral hemisphere, lead to greater cerebral I/R injury.^[16]

Antigens released from ischemic brain tissue bind to the pattern recognition receptor of splenic macrophages to activate them. Activated macrophages transmit signals through myeloid differentiation factor 88 to induce nuclear translocation of nuclear transcription factor kappa B (NF- κ B).^[17] NF- κ B-dependent transcriptional activity triggers the expression of factors that coregulate the inflammatory response. In peripheral inflammatory cells, macrophages are the main innate immune cells that activate adaptive immunity and release pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-8, and MCP-1. These promote the migration and infiltration of inflammatory cells from the spleen to the brain and aggravate inflammatory injury in ischemic brain regions.^[3] Anti-inflammatory approaches which inhibit the release of splenic macrophages are expected to reduce the release of splenic inflammatory cells, leukocyte brain infiltration, and inflammatory brain injury.^[18]

Splenectomy and Other Methods of Spleen Removal after Stroke

Splenectomy is commonly used for spleen suppression in animal studies.^[11] In ischemic mice, the serum levels of proinflammatory factors after splenectomy were lower than in normal mice. Myocardial macrophage infiltration and the expression of inflammatory factors were decreased, and cardiac function after myocardial ischemia was significantly improved by splenectomy.^[19] Splenectomy 2 weeks, or immediately before or after stroke reduced infarct volume and inflammatory cell infiltration after reperfusion in ischemic rats.^[11,20,21] These studies suggested that splenectomy inhibited the secondary inflammatory injury induced by the release of splenic inflammatory cells. Pulsed ultrasound and radiotherapy of the spleen have been used as noninvasive treatments for inhibiting spleen inflammatory reactions.^[22] However, splenic pulse therapy damaged the septic immune system in rats by activating β 2 adrenergic receptors in the cholinergic anti-inflammatory pathway.^[12] Although it reduced infarct volume, radiation of the spleen after stroke in rats caused apoptosis of splenic immune cells.^[12] Importantly, splenectomy, while acutely reducing infarct volume, did

not improve long-term functional outcome in rats.^[23] This was attributed to a disturbance of the T-to-B cell ratio. Furthermore, splenectomy resulted in host defense deficiency, a tendency to severe pyogenic bacterial infection, and increased risk of thrombosis.^[24] The response of inflammatory cells to stroke is dynamic^[13] where the proportion of inflammatory cells in proinflammatory and anti-inflammatory phenotypes (i.e., M1 and M2 macrophages) change at different stages of stroke.^[25] The decrease of inflammatory cells caused by permanent splenic suppression induces a state of immunosuppression and increases the risk of infection.

Taken together, obviously, splenectomy, or other methods that permanently remove spleen function are not practical in a clinical setting. It is thus desirable to consider means to inhibit spleen function that are temporary and reversible. Reversible inhibition of spleen function after stroke could be used to inhibit pro-inflammatory spleen actions during acute stroke, but would not impede long-term spleen function involved with tissue repair. Such methods could potentially serve as clinical treatments to mitigate stroke damage.

The Key Role of Spleen Immune Response in Ischemia/Reperfusion Injury

The complex, redundant, and overlapping effects of inflammatory cells and their mediators are highlighted in the poststroke immune response.^[26] Polymorphonuclear leukocyte infiltration in areas of cerebral infarct, and cytokine-mediated inflammatory reactions, play a pivotal role in reperfusion injury in both experimental animal models and stroke patients.^[27,28] IL-1 β and TNF- α , produced by endothelial cells, astrocytes, fibroblasts, macrophages, and neurons, are the key cytokines that fuel inflammatory reactions in the brain. Leukocytes potentiate reperfusion brain injury by (1) clogging the microcirculation, leading to the so-called “no flow” phenomenon, and (2) by infiltrating into the parenchyma, leading to inflammation. Upregulation of these inflammatory mediators, as well as blood-borne inflammatory cell adherence and infiltration, results in enhanced permeability of brain endothelium and blood-brain barrier disruption.^[29-31]

Future Direction on Regulation of Splenic Function after Stroke

The changes in the spleen after stroke are not only reflected in morphology but also, more importantly in immune cells and cytokines, which are closely related to stroke outcomes. These processes include activation of the autonomic nervous system, release of central nervous system antigens, and chemokine/chemokine receptor interactions.^[32] Therefore, the regulation of spleen immunity is the key to regulating spleen function.

Previous studies regarding the intervention of spleen function through stem cell transplantation have proved that the spleen is a potential target of various stem cells, such as multipotent adult progenitor cells. When systemically administered, many therapeutic stem cells migrate to the spleen after brain injury, so modulating peripheral immune status after stroke may be a potentially important therapeutic strategy, especially for improving the long-term outcome of stroke patients. Exosomes derived from stem cells have been found to have therapeutic effects on hemorrhagic stroke.^[33] All types of stem cells and exosomes have different effects on anti-inflammatory pathways such as IL, interferon, and cholinergic pathways in the spleen after stroke. These issues will be focused on in future studies.

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Conflicts of interest

Dr. Yuchuan Ding is an Associate Editor, Dr. Xiaokun Geng is an Editorial Board member of *Brain Circulation*. The article was subject to the journal’s standard procedures, with peer review handled independently of them and their research groups.

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