Inhibiting the formation of neutrophil extracellular trapping: a potential mechanism of Chinese medicine in the treatment of ischemic stroke

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Abstract

Ischemic stroke (IS) is the main killer that endangers the health and life of middle-aged and elderly people worldwide. Inflammatory response plays a key regulatory role in the pathogenesis of IS. After cerebral ischemia, leukocytes rapidly accumulate, penetrate blood vessels and infiltrate brain tissue, thereby activating pro-inflammatory factors in the infarct area to exacerbate nerve damage. Neutrophil extracellular traps (NETs) are fibrous mesh structures released by activated neutrophils outside the cell, which can clear pathogens and cell debris, induce inflammatory responses and exacerbate cerebral ischemia-reperfusion (CI/R) injury. Various traditional Chinese medicines and their main components can improve neurological function defects after IS, and inhibit the formation of NETs, which opens up a new direction for the study of traditional Chinese medicines in the prevention and treatment of IS.

Keywords: neutrophil extracellular traps; Ischemic stroke; Inflammatory response; traditional Chinese medicine

Author contributions

Huan-tian Cui and Wei-bo Wen conceived this study, Yao Chen carried out this study, and drafted the manuscript. Lei Feng, Qin-zhao Zhang and Chen Yang collected and analyzed these materials, Yan-fang Zheng, Lu Gao and You-xiang Cui were responsible for this manuscript and reviewed the article critically. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

IS, Ischemic Stroke; NETs, neutrophil extracellular traps; CI/R, cerebral ischemia-reperfusion; ROS, reactive oxygen species; MMPs, matrix metalloproteinasases; NO, nitric oxide; MPO, myeloperoxidase; NE, neutrophil elastase; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, nitric oxide synthase; PAD4, peptidase-serine-arginine deaminase IV; PMA, phorbol 12-myristate 13-acetate; ERK, extracellular signal-regulated kinase; GSDMD, Gasdermin D; CitH3, Citrulline Histone H3; AS, Atherosclerosis; ox-LDL, oxidized low-density lipoprotein; dsDNA, double-stranded DNA; CitH4, Citrulline Histone H4; FXII, coagulation factor XII; r-tpa, recombinant tissue-type plasminogen activator; TCM, traditional Chinese medicine; LOX-1, Oxidized Low Density Lipoprotein Receptor 1; PNS, panax notoginseng saponins; MCAO/R, focal cerebral ischemia/reperfusion; Ly6G, Lymphocyte antigen 6G; ISO, Isopenaline.

Citation

Introduction
Stroke is the third leading cause of disability and death worldwide [1]. According to reports, there were 101 million stroke cases worldwide in 2019, including 12.2 million new cases and 6.55 million deaths due to stroke [2]. Among them, IS accounts for about 62.4% of all types of stroke [3]. IS can occur at any age, with an average age of onset of about 65 years in China, significantly lower than the 75 years in developed countries [4]. Stroke, as a disease with high incidence rate and high mortality, seriously affects the quality of life of patients and brings heavy economic burden to families and society [5]. In recent years, the rapid progress of IS research and the emergence of intravenous thrombolytic drugs and mechanical thrombectomy have greatly improved the clinical symptoms of IS patients and prolonged their survival time. However, due to the limitations of time window, indications, and treatment costs, many patients cannot achieve early vascular recanalization and cerebral tissue reperfusion through intravenous thrombolysis and mechanical thrombectomy, resulting in severe disabilities such as hemiplegia, language impairment, and visual impairment. In addition, there is currently no conclusive evidence in clinical trials that neuroprotective agents are beneficial for acute ischemic stroke. The treatment of language and physical disabilities caused by stroke has become a worldwide problem. Therefore, more in-depth research is needed on the pathogenesis of IS to identify new therapeutic targets.

Inflammatory reaction runs through the whole process of IS
The pathogenesis of IS is due to atherosclerosis, cerebral thrombosis and other cerebrovascular diseases, resulting in local vascular lumen stenosis or occlusion, leading to ischemic and hypoxic necrosis of brain tissue [6]. IS is an intricate and dynamic pathophysiological process. The occurrence of cerebral ischemia promotes a series of cascading reactions in brain tissue cells, including energy metabolism disorders, excitatory amino acid toxicity, oxidative stress damage, inflammatory response, and cell apoptosis. Each link influences each other, ultimately leading to irreversible damage to brain tissue [7]. Among them, inflammatory response runs through the entire pathological process of IS, reaching its peak hours to days after ischemia [8], is one of the main factors affecting the prognosis of ischemic stroke. Inflammatory factors can not only directly cause brain tissue damage, but also stimulate the production of reactive oxygen species (ROS) and induce the activation and infiltration of inflammatory cells [9], including the activation of microglia and astrocytes, as well as the infiltration of neutrophils and T lymphocytes from the periphery to the central nervous system [10]. Infiltrating leukocytes produce pro-inflammatory cytokines, matrix metalloproteinases (MMPs), nitric oxide (NO), ROS, and other cytotoxic factors, which accelerate brain injury [11].

Neutrophils form extracellular traps to promote cell damage
Neutrophils are the main defensive cells of the body, which clear invading pathogens through phagocytosis, degranulation, and release of neutrophil extracellular traps; during sterile tissue injury, neutrophils also participate in the clearance of cellular debris, restoring tissue homeostasis [12]. NETs are fibrous networks released into the extracellular space during the process of neutrophil-initiated inflammatory cell death, consisting of double-stranded DNA, histone, and granular protein. The core histones are H2A, H2B, H3, and H4, and the granular proteins mainly include myeloperoxidase (MPO) [13] and neutrophil elastase (NE) [14]. The formation mechanism of NETs is as follows: the nuclear membrane and granular membrane disintegrate and release granular proteins such as NE and MPO [15], NE can alter the cytoskeleton of neutrophils and enter the nucleus to work synergistically with MPO to cause chromatin decompensation [16]. Through nicotinamide adenine dinucleotide phosphate (NADPH), nitric oxide synthase (NOS), or other mechanisms, it causes peptide-serine-arginine deaminase IV (PAD4) to convert arginine residues into citrulline residues, resulting in histone deamination, loss of positive charge, and chromatin decompensation [17]. Autophagy, a process of degradation and recycling of cellular components, is also involved in the formation of NETs.

Recently, the pathogenic characteristics of NETs have attracted great attention. When stimuli persist, an imbalanced immune response may lead to the deregulation of NET release, thereby exacerbating inflammation and causing host tissue damage beyond anti-inflammatory functions [18]. The release of NETs in the human body mainly occurs in the form of suicidal NETosis, in which neutrophils are activated by phorbol 12-myristate 13-acetate (PMA) by upregulating the glycosylation pathway, inducing the activation of extracellular signal-regulated kinase (ERK), and subsequently inducing the phosphorylation and assembly of NADPH oxidase complexes. NADPH oxidase can regulate the production of ROS [19], increase Ca2+ levels, and further activate PAD4. Activated PAD4 converts arginine in histone H3 and H4 into citrulline, leading to a loss of cations in histones and the condensation of chromatin [20]. At the same time, the massive production of ROS can destroy the granular membrane and lysosomal membrane of neutrophils, inducing the release of MPO and NE. NE can convert GSDMD into an active form, GSDMD-NT, mediating the formation of pores in the nuclear membrane, granular membrane, and plasma membrane. In this process, granular proteins are transferred to the nucleus to participate in chromatin unfolding. Subsequently, the nuclear membrane disintegrates, chromatin depolymerizes, the plasma membrane disappears, the nuclear and cytoplasmic substances mix, cytoplasmic organelles disappear, and finally DNA-based NETs spill over into the extracellular space [21–22].

NETs promote the occurrence and development of IS
NETs have been shown to be closely associated with the pathological processes of IS. Animal studies have found that there is a significant infiltration of neutrophils in infarcted brain tissue, and the infiltration of neutrophils is more pronounced in chronic infarcts than in acute infarcts. At the same time, there is a significant expression of NETs in the brain parenchyma and peripheral blood [23]. In the rat permanent middle cerebral artery occlusion-reperfusion model, it was also found that neutrophils injured by ischemia rapidly infiltrated into the damaged brain tissue and exacerbated inflammation. Subsequently, CitH3 was detected in the leptomeninges, striatum, and brain parenchyma at different time points, confirming the presence of NETs [23].

NETs promotes the development of atherosclerotic plaque
Atherosclerosis (AS) is a lipid driven vascular inflammatory disease, which is an important cause of ischemic heart disease and stroke. When the vascular intima is damaged, monocytes in the blood enter the subintima and transform into macrophages. After ingesting a large amount of oxidized low-density lipoprotein (ox-LDL), foam cells and lipid stripes are formed. At the same time, ox-LDL promotes macrophages to secrete a large amount of inflammatory mediators, triggering vascular wall inflammation, accelerating the development of AS, accelerating the rupture of unstable plaques in AS, and ultimately leading to the occurrence of ischemic cardiovascular and cerebrovascular diseases [24]. NETs are involved in the formation of AS. Some studies have found that NETs exist in atherosclerotic plaques in humans and mice [25], which is related to the type and prognosis of plaque, and can become a promising biomarker and therapeutic target [26]. The double-stranded DNA and histones in the NETs structure provide a fulcrum for the binding of platelets and red blood cells, IL-1α and cathespin G can damage vascular endothelial cells, enhance thrombosis of endothelial cells and induce platelet activation, promote immune inflammation-related reactions, and thus aggravate AS formation [27]. Borisoff JI et al. detected the levels of NETs related markers (including dsDNA, nucleosome, CitH4 and MPO-DNA) in patients with coronary atherosclerosis, and found that NETs is associated with severe AS [28].

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**NETs promote blood coagulation and thrombosis**

NETs can induce thrombosis in the blood to prevent the spread of infectious pathogens. However, excessive NETs production can mediate microvascular thrombosis, induce endothelial cell death, and lead to the occurrence of cerebrovascular disease [29]. NETs can stimulate exogenous and endogenous coagulation pathways to participate in thrombosis. NE in NETs stimulates the exogenous coagulation pathway by binding and proteolytic inhibition of inhibitors of the exogenous coagulation pathway [30], and can also activate FXII through the endogenous coagulation pathway to participate in thrombosis [31]. DNA in NETs has the effect of enhancing the coagulability of serine proteases [32], and histone indirectly promotes blood coagulation by activating platelets and stimulating platelet granule release of prothrombin-activating polypeptide [33]. At the same time, histone has an inhibitory effect on anticoagulants in the blood, and can interact with thrombomodulin and protein C to inhibit the activation of thrombomodulin protein C. Plasma thrombin generation is dose-dependent on histone [34]. In addition, NETs stimulate thrombosis by providing a DNA skeleton; by capturing red blood cells and platelets, binding fibrinogen, fibronectin, von Willebrand factor, and tissue factor, NETs promote the formation and stability of blood clots. A recent study on the composition of emboli in acute cerebral infarction with poor response to t-PA therapy found that the platelet-rich region of arterial thrombi is mainly composed of platelets and fibrin, and there are also a large number of NETs, thus NETs are considered to be the main participants in immune thrombosis [35] (Figure 1).

**Traditional Chinese medicine inhibits the formation of NETs**

The treatment of IS with traditional Chinese medicine (TCM) is not only rich in resources and effective, but also has few side effects and multiple components and targets, making it a hot topic for scientists at home and abroad. However, due to the uncertainty of specific active ingredients and mechanisms of action, the use and promotion of traditional Chinese medicine have been limited. The generation of NETs is closely related to the occurrence and progress of atherosclerosis, arteriovenous thrombosis, ischemia reperfusion and other ischemic vascular diseases [36, 37, 38]. Inhibiting the formation of NETs has become a potential target for prevention and treatment of IS. Salvia miltiorrhiza has the effects of promoting blood circulation, removing blood stasis, dredging channels and relieving pain. It is mainly used in clinical treatment of ischemic cardio cerebrovascular diseases. Its main active ingredient tanshinone IIA can significantly improve the area of atherosclerotic plaque in ApoE -/- mice, reduce the levels of serum low-density lipoprotein and triacylglycerol, inhibit the activation of oxLDL on macrophage LOX-1 and CD36, and interfere with NF-κB Pathway, which reduces the activation of the downstream NLRP3 inflammasome and reduces IL-1β, IL-18, TNF-α and IL-6 levels [39]. Tanshinone IIA can also inhibit the production of inflammatory factors by neutrophils by regulating NETs formation, prevent the release of MPO and NE, and reduce NETs formation, thereby alleviating inflammatory reactions [40]. Buyang Huanwu Decoction is a traditional Chinese medicine compound used to treat or prevent ischemic stroke. It can inhibit platelet aggregation, protect vascular endothelial cells, reduce the formation of thrombin and related proteins, effectively inhibit the formation of NETs, control inflammatory reactions [41], and reduce the volume of cerebral infarction [42] after cerebral ischemia-reperfusion. Sanqi has the effects of removing blood stasis, promoting blood circulation, and relieving pain. Its main component, panax notoginseng saponins (PNS), not only significantly reduces the neurological function score of MCAO/R rats, reduces the volume of cerebral infarction, and significantly reduce the levels of IL-1β, TNF-α, and IL-6 in brain tissue [43]. It can also downregulate the expression of Ly6G and MPO in plaque and Ly6G and C/β-H3 in myocardial areas, inhibit the activation of NETs, reduce the plaque area and lipid deposition in the plaque of ISO mice, and improve myocardial injury in ISO mice [44]. Other traditional Chinese medicines or traditional Chinese medicine compounds, such as magnolol [45] and Huanglian Jiedu Tang [46], can improve focal cerebral ischemia-reperfusion injury, inhibit inflammatory response, and reduce NETs formation. These may have potential mechanisms for inhibiting NETs to treat IS, but there is a lack of relevant research reports.

**Outlook**

NETs play an important role in ischemia-reperfusion injury. Although they have a positive effect on the clearance of pathogens, excessively generated NETs can also promote the occurrence and development of ischemia-reperfusion injury. Effectively inhibiting inflammatory response and nets generation is rapidly becoming a new target for the treatment of IS. However, due to the limitations of current research, the specific mechanisms of NETs and the interactions between NETs and IS, including the inflammasome and autoaphagy, remain unclear. Therefore, studying the pathogenesis of NETs as a key target can provide new ideas and directions for drug development and precision.

![Figure 1 The association of NETs and IS](image-url)
diagnosis and treatment of IS. Although traditional Chinese medicine has achieved definite therapeutic effects in treating IS and can significantly improve the symptoms of neurological deficits, there is a lack of evidence on the regulatory effects of traditional Chinese medicine and its compound preparations on NETs, as well as clinical trials. Therefore, further research is needed to investigate the mechanism of action of traditional Chinese medicine as a NETs inhibitor.

References


