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The role of neutrophils in the pathogenesis of ischemic stroke

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ABSTRACT

Background. Immune responses and inflammation play an important role in the pathogenesis of ischemic stroke.

Aim. To analyze the involvement of neutrophils in the pathogenesis of ischemic stroke.

Results. Data on the contribution of neutrophil granulocytes to the development of local sterile inflammation and secondary brain injury in acute ischemic stroke were summarized. Mechanisms of neutrophil influence on thrombosis, neutrophil extracellular trap formation (NETosis), protease release, and direct interaction with platelets with subsequent formation of platelet-leukocyte aggregates were discussed. Available information on the effectiveness of reperfusion therapy and an association of changes in neutrophil activity with development of infectious complications of stroke were presented. In addition, research data were presented on the contribution of neutrophils to atherogenesis, which is one of the most important etiological factors in ischemic stroke. The review showed that the contribution of neutrophils to the pathogenesis of ischemic stroke is associated with implementation of their secretory, regulatory, and phagocytic functions, as well as with NETosis.

Conclusion. It was shown that neutrophils are involved in the pathogenesis of ischemic stroke and modulate a response to treatment.

Key words: neutrophils, neutrophil extracellular traps, NETosis, ischemic stroke, post-stroke infections, thrombolysis.

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Роль нейтрофилов в патогенезе ишемического инсульта

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РЕЗЮМЕ

Актуальность. Иммунные реакции и воспалительный процесс играют важную роль в патогенезе ишемического инсульта.

Цель. На основании научных публикаций проанализировать вовлеченность нейтрофилов в патогенез ишемического инсульта.

Результаты. Обобщены данные о вкладе нейтрофильных гранулоцитов в развитие локального асептического воспаления и вторичного повреждения мозга при остром инсульте. Обсуждены механизмы влияния нейтрофилов на процесс тромбообразования, образование нейтрофильных внеклеточных ловушек (нетоз), высвобождение протеаз и прямое взаимодействие с тромбоцитами с образованием лейкоцитарно-тромбоцитарных агрегатов. Приведены имеющиеся сведения об эффективности реперфузионной терапии, а также ассоциации изменений активности нейтрофилов с развитием инфекционных осложнений инсульта. Представлены данные исследований о вкладе нейтрофилов в атерогенез, являющийся одним из важнейших этиологических факторов ишемического инсульта. Показано, что участие нейтрофилов в патогенезе ишемического инсульта связано с реализацией их секреторных, регуляторных, фагоцитарных функций и с нетозом.

Заключение. Установлено, что нейтрофилы принимают активное участие в патогенезе ишемического инсульта и модулируют ответ на лечение.

Ключевые слова: нейтрофилы, нейтрофильные внеклеточные ловушки, нетоз, ишемический инсульт, инфекционные осложнения инсульта, тромболитическая терапия.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования.

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INTRODUCTION

Stroke is one of the leading causes of mortality and persistent disability among the population both in the Russian Federation and in the world. About 450,000 stroke cases per year are registered in the Russian Federation [1]. The average rate of death during the first 30 days after stroke, according to different authors, ranges from 17 to 34%. Only half of patients who have experienced the first stroke live for 3 years or more, and 35% of the patients live 10 years or more [2]. In addition to mortality directly from stroke, a large number of deaths are caused by its infectious and inflammatory complications, primarily pneumonia [3, 4]. In more than 80% of patients, stroke leads to disability with persistent neurological deficiency [5].

Immune responses and inflammation initiated by ischemia are important pathogenetic phenomena causing destruction of the brain tissue [6]. It is assumed that features of the inflammatory response closely associated with depletion of antioxidant mechanisms and impaired microcirculation and blood – brain barrier (BBB) largely determine a course of the disease at all its stages [7]. At the same time, a dual role of immune system activation in acute stroke is noted.

On the one hand, the immune response is sanogenetic and aimed at removing necrotic tissue. On the other hand, it is able to aggravate the course of ischemic stroke, increasing the area of infarction [8]. During neuronal death, under the influence of gene expression, synthesis of proinflammatory cytokines (interleukin (IL)-1 α and IL-1β, tumor necrosis factorα (TNFα)), cell – cell interaction molecules, and cyclooxygenase-2 is activated [9, 10]. The cascade of reactions initiated by ischemia inevitably involves factors of innate immunity, including the largest population of leukocytes – neutrophils.

From the moment I.I. Mechnikov discovered phagocytosis of microorganisms by neutrophil granulocytes in the 19th century, views on their role in immunological processes have changed significantly [11–14]. To date, basic research has convincingly

proved that the role of neutrophils is not limited to elimination of extracellular pathogens [13, 14]. They also perform a regulatory function by activating and modulating adaptive immunity, contributing to its full implementation [15]. Neutrophils, in the light of modern perceptions, are a pluripotent population of cells of the immune system related to innate immunity and playing an important role in maintaining immune homeostasis in the body [16].

Neutrophil population is heterogeneous and includes different subpopulations of cells [17]. Depending on mediators, receptors, and markers produced, they are divided into proinflammatory, inflammatory with antimicrobial potential, inflammatory with negative cytotoxic potential ("aggressive"), anti-inflammatory – regulating inflammation regression, anti-tumor, and hybrid, combining the properties of neutrophils and dendritic cells [13, 14]. A study of neutrophil subpopulations in inflammatory diseases revealed their predictive significance as markers of the severe course of acute infection and its adverse outcome [11].

In recent years, a large amount of new information has appeared that neutrophils are involved in the pathogenetic mechanisms of many noncommunicable diseases, including vascular ones [4, 13]. In this regard, it is of great interest to study the role of neutrophils in the development of ischemic stroke, which is one of the least studied aspects in the immunology of cerebrovascular disorders.

The aim of this review was to analyze currently available data on the role of neutrophils in the pathogenesis of ischemic stroke.

THE ROLE OF NEUTROPHILS IN STERILE INFLAMMATION IN CEREBRAL ISCHEMIA

Neutrophils are some of the most fast-reacting cells in the immune system. They migrate to the brain within minutes from the onset of stroke. The mechanism of intrathecal neutrophil entry in ischemic injury was studied in experimental models with lifetime imaging. Neutrophils migrate along the vessels even against the blood flow in order to exit the vascular bed and enter the infarct zone [18]. Neutrophils are capable of expressing various endothelial adhesion molecules during the first 15 minutes after ischemia [19].

Afterwards, 6–8 hours after stroke, neutrophils surround cerebral vessels and initiate infiltration of surrounding tissue [20, 21]. In experimental models of ischemic stroke, it was shown that infiltration with neutrophils occurs already on the first day, reaching a peak on day 3, and then begins to decrease, but is present both 7 and 15 days after the development of cerebral ischemia.

M. Gelderblom et al. (2012) demonstrated that neutrophils are predominant immune cells in the ischemized hemisphere 3 days after middle cerebral artery occlusion in the experiment [22]. R.M.Weston et al. (2007) reported that neutrophil infiltration of ischemized tissues persists for up to 32 days, but the presence of neutrophils is disguised by the concentration of activated macrophages / microglia [19].

Penetrating into the ischemic area, neutrophils cause secondary damage to the brain tissue due to the release of proinflammatory factors, reactive oxygen species, proteases, and matrix peroxidases [23]. These factors damage the endothelial cell membrane and basal plate, leading to increased BBB permeability and post-ischemic brain edema [24].

NEUTROPHILS AND THROMBOSIS

The ability of neutrophils to participate in thrombosis through various mechanisms, such as formation of neutrophil extracellular traps (NETosis), release of proteases, and direct interaction with platelets to form leukocyte – platelet aggregates, deserves particular attention. An increase in the number of such aggregates was detected in the blood of patients with symptomatic carotid artery stenosis [25]. A decrease in the intensity of platelet – neutrophil aggregate formation occurs with inhibition of receptors to glycoprotein IIb / IIIa and selectins. The formation of leukocyte – platelet aggregates in acute ischemic stroke and reperfusion therapy can become a useful biomarker and target for treatment, since these aggregates increase intravascular thrombosis [26].

Due to great mobility, neutrophils, ahead of platelets, bind to the activated endothelium of damaged vessels using ICAM-1 receptors. This leads to activation and accumulation of platelets in the damaged vessel and an increase in thrombosis. There are a number of studies aimed at exploring possible ways to prevent this process [27, 28].

Proteases and cathepsin G, released by neutrophils, interact with coagulation factors, accelerating thrombus formation. Inhibition of cathepsin G in an experimental mouse model demonstrated improvement in the outcome of ischemic stroke. On the other

hand, protease ADAMTS13 released by neutrophils cleaves highly active von Willebrand factor and thereby reduces the activity of inflammation in the acute phase of ischemic stroke [29].

The prothrombotic activity of neutrophils is also explained by their ability to release molecules involved in the formation of a neutrophil extracellular trap (NET), release proteases, and directly interact with platelets. These processes can lead to increased brain ischemia in stroke [30].

NETs are formed in response to various stimuli, the key mechanism for their formation is activation of peptidylarginine deiminase 4 (PAD4). This enzyme is necessary for histone citrullination, which leads to chromatin decondensation, as a result of which separate strands of DNA are formed. Apart from DNA strands, the structure of NETs includes histones and specific protein granules, such as elastase and myeloperoxidase of neutrophils. This makes it possible to create a locally high concentration of cytotoxic substances in the DNA area. Initially, formation of NETs was known to be associated with their bactericidal effect [31], but lately, they have been increasingly referred to as the key participants in thrombosis [32].

Platelets can bind to released NETs and be activated due to their association with histones [33, 34]. Interaction with neutrophils occurs through the association of platelet P-selectin with neutrophil P-selectin glycoprotein ligand [35]. When activated, platelets are able to release HMGB-1 protein and present it on their surface, increasing neutrophil release of new DNA networks [36]. Histones associated with NETs also increase thrombin generation by platelets [35] and provoke the release of von Willebrand factor from Weibel – Palade bodies in endothelial cells, leading to leukocyte – platelet adhesion [37]. This process potentiates itself by activating the extrinsic coagulation pathway and leads to further thrombosis and inflammatory response in stroke [38].

The prothrombotic activity of NETs is also determined by their polyanionic surface, which activates the coagulation factor XII, participating in the activation of the intrinsic coagulation pathway [39]. The strategy of controlling the deoxyribonuclease activity in experimental models of stroke in mice resulted in dissolution of NETs and a protective effect *in vivo* [40].

Based on the accumulated data, it is assumed that the prothrombotic activity of neutrophils can both increase the risk of ischemic stroke and contribute to further thrombosis in the acute phase of cerebrovascular disorder.

NEUTROPHILS AND SYSTEMIC THROMBOLYTIC THERAPY FOR STROKE

Currently, intravenous systemic thrombolysis using the recombinant tissue plasminogen activator (p-TAP) is a recognized and recommended method for treating patients with ischemic stroke, but its use is associated with an increased risk of intracranial hemorrhage [41], an inflammatory response in the brain tissues [42], and infiltration of the brain tissues with neutrophils [43]. These factors influence clinical effectiveness of thrombolytic therapy in ischemic stroke [44].

A number of studies investigated the role of neutrophils in the therapy using p-TAP [45]. It was shown that p-TAP therapy can affect the dynamics of changes in the neutrophil count in the brain tissues in stroke [44, 46]. There is evidence that an increase in the neutrophil count in peripheral blood in the first 24 hours after thrombolytic therapy is associated with an unfavorable prognosis [46]. This pattern may be explained by the formation of NETs. Thus, in acute myocardial infarction, an increase in the number of NETs in thrombolytic therapy was associated with a worse functional outcome and identified NETs as a promising therapeutic target for improving the effectiveness of p-TAP therapy [47].

Some studies note that when p-TAP and recombinant deoxyribonuclease I (DNAse I) are used together to inhibit NETs, the outcome of thrombolytic therapy is improved; the duration and number of attempts during further endovascular reperfusion therapy are reduced compared with the use of p-TAP alone [46]. Similar conclusions were made in a number of studies which examined the possibility of potentiating p-TAP with DNAase I in thrombolytic therapy of acute myocardial infarction and ischemic stroke [48, 49]. In addition, the ability of extracellular DNA and histones to modify fibrin structure, making it more resistant to mechanical and enzymatic effects, can be a possible reason for correlating the increase in the number of NETs with worse results of thrombolytic therapy [50].

Therefore, the study of neutrophil activity in thrombosis is of interest for further optimization of reperfusion therapy for acute ischemic stroke and development of new methods for treatment and prognosis in this disease.

NEUTROPHILS AND INFLAMMATORY COMPLICATIONS OF STROKE

Infectious inflammatory diseases are some of the most common and dangerous complications of stroke, which significantly affect the survival and recovery of patients [51]. In order to ensure optimal homeostasis in the body, nervous and immune systems are closely connected by multiple pathways, and serious damage to the brain tissue in stroke leads to multiple changes in these neuroimmune interactions [52].

Ischemized brain tissue affects peripheral immune cells, suppressing their activity, which is further manifested through lymphopenia, a decrease in proinflammatory cytokine production, and reduced monocyte activity [53]. This mechanism is probably adaptive and aimed at reducing the intensity of the inflammatory response in the ischemic area and lowering a risk of autoimmune diseases in response to neuroantigens under conditions of increased BBB permeability [54]. On the other hand, this leads to development of secondary immunodeficiency and can contribute to development of infectious complications [55]. The size of the ischemic area is an independent risk factor for immunosuppression and inflammatory complications after stroke [56].

In addition, development of secondary immunodeficiency in acute stroke is mediated by changes in the autonomic nervous system activity and hypothalamic – pituitary – adrenal (HPA) axis [57]. Mediators in this process are noradrenaline, acetylcholine, and glucocorticoids receptors to which are widely presented on cells of the immune system [58]. It is known that increased production of glucocorticoids upon activation of the HPA axis induces apoptosis in immune cells [59].

It should be noted that clinical studies on the role of neutrophils in the development of infectious complications of stroke are few. In one of them, conducted at the Hannover Medical School (Germany, 2015–2017), an attempt was made to determine an association between decreased neutrophil granulocyte function and the emergence of inflammatory complications in the acute phase of stroke.

The examination of 95 patients, no significant changes in the phagocytic activity of neutrophils were detected. However, peripheral blood neutrophils in the group of patients with early (first 7 days) infectious complications decreased their ability to release reactive oxygen species in response to N-formyl-methionyl-leucyl-phenylalanine (FMLP) stimulation, while maintaining the ability to release reactive oxygen species when stimulated by more active inducers, for example, *E. coli*.

In a clinical trial with anti-ICAM-1 monoclonal antibodies (Enlimomab) inhibiting neutrophil function and reducing the cerebral infarct volume in an experimental model, an increased incidence of infections, especially pneumonia, was observed. These inflammatory processes were associated with worsening of a stroke outcome, indicating a negative effect of neutrophil dysfunction in the development of infectious complications [61].

NEUTROPHILS AND ATHEROGENESIS

Apart from the direct impact on all stages of the ischemic cascade and the risk of developing infectious complications, neutrophils are actively involved in the processes that lead to stroke. Recently, more attention has been paid to the study of the role of neutrophils in the processes of atherogenesis [62, 63].

There is evidence that neutrophils synthesize and release cholesterol-binding peptides and proteins under conditions of close cell – cell interaction and / or stimulation with proinflammatory cytokines [64]. The ability of venous blood neutrophils to release protein – lipid complexes when cultured under conditions of close cell – cell interaction was called the lipid-releasing capacity of leukocytes (LRCL) [63, 64]. The following studies proved the clinical role of an increase in lipid-releasing capacity of leukocytes and its association with the course of coronary artery disease (CAD) [65].

The role of neutrophils in the development of atherosclerotic lesions is impelemted in several directions. On the one hand, they are involved in early stages of atherogenesis, namely, in the development of endothelial dysfunction. On the other hand, neutrophils affect the emergence of complications in atherosclerosis: destabilization of the atherosclerotic plaque and formation of blood clots [66, 67].

When the vascular endothelium is damaged, fragments of the extracellular matrix, such as hyaluronan, fibronectin, etc., are released into the intercellular space and act as endogenous danger-associated molecular patterns (DAMPs). They are recognized by neutrophil pattern recognition receptors (TLR2/4) in the bloodstream and contribute to their attraction to

the focus of inflammation. In addition, chemotaxis and migration of neutrophils and other white blood cells are enhanced by production of proinflammatory cytokines, such as IL-1, IL-18, IL-8, chemokines, and cell adhesion molecules, by endothelial cells [68–70].

In the focus of inflammation, neutrophils synthesize cholesterol-binding proteins to form protein – lipid complexes accumulating in the vessel wall. In patients with CAD, neutrophil production of the following molecules was increased: C-reactive protein, von Willebrand factor, lipoprotein, and a brain natriuretic peptide precursor. Moreover, neutrophils in the endothelial lesion cause peroxidation of membrane lipids, DNA strand breaks, and endothelial cell damage [71].

The role of neutrophils in the atherogenesis may be also related to their ability to form NETs: extracellular network-like structures and released enzymes lead to endothelial damage [66, 71].

CONCLUSION

Therefore, neutrophils, being a pluripotent heterogeneous cell population, take an active part in the pathogenesis of ischemic stroke and are also able to modulate the response to treatment. Their further study may open up additional prospects for personalization of therapy and prediction of disease outcomes.

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