·Reviews·

Neuroinflammation after acute ischemic stroke: a volcano hard to contain

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【关键词】 脑缺血； 炎症； 中枢神经系统； 免疫，细胞； 神经免疫调节； 综述

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Abstract

Many endogenous, exogenous and systemic factors individually can lead to occlusive vessel pathology affecting the neuroendovascular unit compromising cerebral blood flow. The brain ischemia thus created irrespective of the pathology has similar endpoint and involves blood vessels, astrocytes, neurons and surrounding microglia triggering the whole spectrum of neuroinflammation which is an important component in the ischemic cascade. The resultant series of neuroinflammatory reactions cause depolarization of neuronal cells and activation of pro-inflammatory cellular agents and subsequently cell death. Neuroinflammation can be an effect and (or) cause of acute energy failure, excitotoxicity, ionic imbalance, channel dysfunctions, and oxidative free radicals in the central nervous system. Non-restoration of the blood flow within the threshold period can result in an extensive brain damage from activation of deadly latent proteases like matrix metalloprotease, and immediate early genes destroying the neuronal microenvironment and blood brain barrier. It is paradoxical that both deoxygenation and reoxygenation can contribute significantly to the stroke neuroinflammatory injury. The cascade of cerebral injury also involves activation of microglia and astrocytes leading to release of chemical mediators like cytokines, oxygen free radicals, neurotoxic and neurotropic factors further contributing to the damage. Neutrophil activation and binding to endothelial surface using adhesion molecules and their subsequent transmigration to the ischemic core will enhance the injury. Monocyte and macrophage will also play a role in brain injury by its release of cytokine and transformation into phagocytes. Strategy to target various players of neuroinflammation to halt or minimize the cerebral damage concentrate on inhibiting intracellular adhesion molecules (ICAMs), vascular cell adhesion molecules (VCAMs), neutrophils, microglia, major histocompatibility complex (MHC), cytokine, chemokine and free radical scavenger system. Different strategies to suppress inflammation secondary to ischemia have shown promise in experimental environment but have failed to demonstrate successful clinical translation and further studies are underway. However, this knowledge of neuroinflammation becomes crucial in developing novel therapies for neuroprotection and broadening the field of therapeutic options for cerebral ischemia which is currently limited. In this brief review we try to address diverse mechanisms involving neuroinflammation in relation to ischemic stroke.

【摘要】 各种内源性、外源性和系统性因素均可影响脑血流的神经血管内单位，但又互为导致血管闭塞的独立因素。无论何种机制引起的脑缺血，其结局均相同，包括血管因素、星形胶质细胞、神经元和诱发神经炎症疾病谱的周围小胶质细胞。而神经炎症反应则为缺血级联反应中的重要环节，可引起神经细胞去极化和炎性细胞因子前体激活，继而细胞死亡。神经炎症反应可引起中枢神

经系统急性能量衰竭、兴奋性毒性反应、电解质紊乱、离子通道功能障碍和氧自由基分泌增多等一系列反应，而又可以是其后果。若在阈值期内缺血状态不能得到改善，则可激活基质金属蛋白酶，导致脑组织广泛损害，而即早基因激活将致使神经体液微内环境和血-脑脊液屏障破坏。脱氧和复氧均可造成卒中相关性损伤。缺血性脑损伤级联反应的病理过程还包括小胶质细胞和星形胶质细胞活化，释放细胞因子、氧自由基、神经毒性因子和神经营养因子等化学介质，进一步加重脑损伤。与此同时，中性粒细胞活化并通过细胞内黏附因子结合到内皮细胞表面，然后移行至缺血核心区，加剧脑组织损伤。单核细胞和巨噬细胞通过释放炎性细胞因子和转化为吞噬细胞而在缺血性脑损伤过程中发挥作用。针对神经炎症不同环节的治疗原则，是停止或减轻脑损伤程度，如抑制细胞内黏附分子(ICAMs)、血管细胞黏附分子(VCAMs)、中性粒细胞、小胶质细胞、主要组织相容性复合物(MHC)、细胞因子、趋化因子和自由基活性。上述针对脑缺血后神经炎症的各种治疗方法，动物实验已有可喜的结果，但临床试验未见成效，目前正在做进一步研究。然而，了解神经炎症的病理学机制，对今后开发新型神经保护治疗方法的开拓脑缺血治疗领域至关重要。本文旨在阐述缺血性卒中相关神经炎症反应的不同病理学机制。

Introduction

Obstruction of cerebral blood flow produces the central "ischemic core" area of critical perfusion and the peripheral "penumbra region" with functionally disabled but still viable brain tissue at risk of infarction surrounding the ischemic core [1]. Since brain is largely dependent on oxidative phosphorylation for the consumption of glucose and oxygen, deprivation of these constituents will trigger the process of ischemic volcano. Within few seconds to minutes of ischemia, series of biochemical, hormonal and oxidative reactions ensues resulting in microvasculature injury and disruption of blood brain barrier. There is excitotoxicity, oxidative damage and spreading depolarization leading to release of proinflammatory agents and disintegration of cell membranes. The extent of cerebral damage is proportional to the degree and duration of ischemic insult as well as brain's capacity to overcome the depolarization [2]. There will be subsequent anaerobic glycolysis secondary to disruption of mitochondrial metabolism in the absence of ATP contributing further to the pathology [3 - 4]. Excitotoxicity and oxidative stress are crucial in the neuroinflammatory process causing blood brain barrier disruption and cell death.

Excitotoxicity and neuroinflammation

The huge energy deficit created by ATP depletion from ischemia release excitatory amino acids which can contribute to the neuronal injury called excitotoxicity. Failure of ATP dependent channels like Na^+/K^+ ATPase and Ca^{2+} pump trigger anaerobic metabolism and elevation of intracellular Ca^{2+} levels in cells. This will lead to extracellular K^+, intracellular Na^+ and lactic acid accumulation creating a disharmonic cerebral environment [5 - 6]. Some believe that accumulation of extracellular K^+ and acidosis are the preceding events in the ischemic cascade leading to ionic disturbances [7]. The cellular efflux of K^+, influx of Na^+ and Ca^{2+} lead to loss of membrane action potential resulting in excessive release of amino acids like glutamate. The extracellular glutamate activates several subtypes of its receptors like N - methyl - D - aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), and metabotropic glutamate receptors [2]. Activation of these glutamate receptors is a very important step in neuroinflammation cascade.

NMDA and metabotropic receptors work through monoionic channels and indirectly result in elevated intracellular Ca^{2+} levels. In contrast, AMPA receptors facilitate Na^+, Cl^- influx and cytotoxic edema via passive influx of H_2O [2]. Multiple pathways including voltage and receptor gated Ca^{2+} influx result in elevation of free cytosolic calcium levels leading to mitochondrial calcium overload; depleting ATP production further [7]. Intracellular Ca^{2+} causes activation of lipases, proteases, kinases, phosphatases, endonucleases and free radicals production [3 - 4, 8 - 9] capitulating phospholipids, protein and nucleic acid breakdown [7, 10 - 13]. NMDA - and AMPA - receptor antagonists may have an important role as a neuroprotective agent via inhibiting the cellular depolarization during the ischemic process [2].

Oxidative stress and neuroinflammation

Ischemic oxidative stress injury arising from imbalance between oxidant and antioxidant production is due to

excessive production of the former resulting in cell death [14 - 17]. Oxidative stress can also play a role in reperfusion injury via several non-enzymatic oxidative reactions when blood flow is restored after a period of scanty cerebral perfusion. Alteration in electron transport system in the mitochondria is the likely source of oxidative stress [18] due to production of free radicals and reactive oxygen species (ROS) on reoxygenation [13, 15, 19 - 20]. Animal studies showed increased formation of superoxide radical anions via alteration in mitochondrial superoxide dismutase (*mSOD*) genes in ischemic injury [21 - 22]. Neuroinflammation produce pro-oxidants leading to elevated intracellular calcium and activation of various receptors and enzymes like NMDA, phospholipases, proteases, nucleases, protein kinases, nitric oxide synthase (NOS) and cyclooxygenase (COX) [16, 23 - 27]. Superoxide free radicals can also generate hydrogen peroxide which eventually forms hydroxyl radical (OH) which again contributes to oxidative damage.

Nitric oxide (NO) is another free radical just like superoxide, but generates from L-arginine by neuronal nitric oxide synthase (nNOS) [19]. NO generated accumulates at toxic levels in reperfusion injury as well as cerebral ischemia leading to neuronal death. NO also participate in inflammatory and cytotoxic reactions in the cascade of cerebral ischemia [28 - 29]. NO can also react with O_2^- free radicals to produce peroxynitrite that subsequently forms peroxynitrous acid. Peroxynitrous acid can directly cause neuronal damage or indirectly via forming free radical and reactive oxygen as well as reactive nitrogen species (RNS) via reacting with other agents in ischemic cascade [30]. At very high concentration NO can also inhibit mitochondrial respiratory chain to facilitate neuronal damage [28]. NO can also facilitate neuronal damage via releasing iron from ferritin [31]. Nuclear factor erythroid 2-related factor 2 (Nrf2) plays a significant role in oxidative stress. It causes transcription of numerous antioxidant proteins and so plays an important role in regulating antioxidant genes by series of enzymatic reactions [32]. These free radicals and ROS are responsible for cell damage via initiating oxidation and (or) peroxidation of cellular lipids, proteins and nucleic acids.

Markers of oxidative stress in stroke

Measurement of oxidative stress can reflect severity of brain damage and help understand the pathophysiology of cerebral ischemia which may aid in developing novel therapeutic approaches in the field of neuroprotective agents [13]. Direct measurement of oxidative stress is difficult and hence the development of several peripheral biomarkers to measure the quantum of oxidative stress. Biological molecules with modified chemical structure produced via reaction of lipid, protein, DNA and ROS can be used as biomarkers [33]. Lipid peroxidation, DNA and protein oxidation along with antioxidants play an important role in generating these biomarkers.

Lipid peroxidation produces malondialdehyde (MDA), 4-hydroxynonenal (HNE), and thiobarbituric acid-reactive substances (TBARs). These aldehydes are known as first biomarkers of oxidative damage [13, 34 - 35]. Oxidative damage to DNA also generates several markers——8-hydroxy-2-deoxy-guanosine (8-OHdG), DNA strand breaks, and DNA-protein cross-links. Most commonly used marker among them is 8-OHdG due to its high specificity and relative abundance in DNA [36]. Protein oxidation on other hand produces advanced oxidation protein products (AOPP) which is an advanced oxidized plasma protein (especially albumin). For protein oxidation, not ample data is available in the field of biomarkers. Studies did not show any difference in protein carbonyls between stroke patients and normal controls [34].

Innate immunity and neuroinflammation in acute stroke

Next step during this process is activation of the pro-inflammatory cells like microglia, astrocytes and leukocytes releasing chemokines, cytokines and other agents like matrix metalloproteinase (MMP).

A microglial cell takes part in phagocytosis but also has capacity to transform into macrophages called activated microglial cells during central nervous system damage. In animal studies, increased number of activated microglial cells was seen up to 16 weeks post 2 hour middle cerebral artery occlusion(MCAO) [37]. These transformed cells produces many cytotoxic agents like several pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6, as well as other potential cytotoxic molecules including NO, ROS, and prostanoids. In addition to cytotoxic agents activated microglial cells also produce neuroprotective agents such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and several other growth factors [38]. Besides microglia, astrocytes also participate in neuro-inflammatory process and produce several cytokines, chemokines, and NO [39]. Besides microglia and astrocytes, leukocytes also have a role in ischemic cascade. Circulatory leukocytes first adhere to the vessel walls and then migrate to extravascular space to produce additional pro-inflammatory mediators. Among all the leukocytes, neutrophils are the first ones to participate in upregulation of gene expression process, infiltrate the area of ischemia and can also secrete cytokine [14].

Cytokines, chemokines and MMP and neuroinflammation in acute stroke

Cytokines are small signaling molecules which can

upregulate the expression of cell adhesion molecules (CAMs) [14]. Elevated intracellular adhesion molecule 1 (ICAM-1) levels in the ischemic core aid leukocytes to migrate into the zone and release cytokines contributing to the blood brain barrier damage. Increased blood brain barrier permeability causes macrophages, T-lymphocytes, natural killer cells, and polymorphonuclear leukocytes migration to the site of ischemic core. Multiple sources including microglia, astrocytes, endothelial cells neurons and peripheral vasculature are responsible for the release of these inflammatory mediators during the process of neuroinflammation [7, 40]. Expression of inflammatory cytokines such as TNF-α, interleukins [IL-1, IL-B, IL-6, IL-20, IL-10 and transforming growth factor-β (TGF-β)] have been studied extensively. It has been shown that IL-1β and TNF-α play an important role as a pro-inflammatory agent in neuronal injury while TGF-β and IL-10 are anti-inflammatory and neuroprotective. These agents are predictors of prognosis after an ischemic injury [14, 41-42]. Chemokines are type of cytokines that participate in migration of peripheral inflammatory cells towards the source of chemokines. MCP-1, macrophage inflammatory protein-1α (MIP-1α), and fractalkine are different types of chemokines. These mediators help in cellular communication and recruitment [14]. MMPs eventually initiate remodeling and degradation of extracellular matrix [7].

Cathepsin G and neuroinflammation

Role for proteolytic enzymes in pathophysiology of stroke has been explored in recent studies [36]. Cathepsin G is a proteolytic enzyme of class serine protease and coded by *CTSG* gene which is expressed in granulocytes, especially neutrophils. Cathepsin G is located in neutrophilic polymorphonuclear leukocytes. It plays a very important role in neuroinflammation via vascular matrix degradation, platelet aggregation, and coagulation disorders [43-44]. Studies have shown the use of serine protease inhibitor as a neuroprotective agent but so far no phase Ⅲ clinical trial available.

Early gene expression and neuronal death

Studies have shown the role of gene regulation in the field of cerebral ischemia. The end point of all the prolonged insult to the central nervous system is cell death. Cell death can be secondary to necrosis or apoptosis depending on type of stimulus, type of cell and stage of that particular cell in life cycle or development. Necrosis is the cause of death when the vascular injury is acute and permanent as seen in ischemic core area. Slower injury as in penumbra zone leads to programmed neuronal death (apoptosis) [45]. Gene expression is one of the crucial steps in the process of apoptosis leading to immediate biochemical changes within minutes of ischemic insult. There are several genes expressing in this process but expression of genes immediately after the ischemia is called as "immediate early genes". Multiple other genes also participating later in the process are named "late response genes", but are less important than early genes. Release of excitatory amino acids such as glutamate, activates series of reactions to modify DNA binding protein which recognize specific binding sites in immediate early genes and regulate gene expression. Modification of these genes plays a significant role in the field of developing novel neuroprotective agents [46] and also against neuroinflammation.

Neuroinflammation and neuroprotective agents in strokes

Currently the use of intravenous recombinant tissue plasminogen activator (rt-PA) is the only medication approved by the Food and Drug Administration (FDA) of USA in the treatment of acute ischemic stroke. However, the use of intravenous rt-PA has limitations, which include a narrow therapeutic time window (within 4.5 hours from symptom onset) and an increased risk of symptomatic intracranial hemorrhage. Additionally, there are several absolute and relative contraindications for its use [47]. Hence it becomes imperative to explore alternative neuroprotective agents.

Several neuroprotective agents have been discovered in the past two decades; however, these agents have been limited to animal models. In the past, studies have targeted glutamate receptors, oxygen free radicals, or sodium and calcium channels in stroke models. While these studies were fairly successful in animal models, it failed in human studies. Earlier trials failed for two reasons: firstly, animal models do not share the same pathophysiology as humans and latter have co-morbidities and risk factors irrelevant for animals; secondly, therapeutic time window varies from species to species [2].

Phase Ⅲ trials have investigated the role of neuroprotective agents in neuroinflammation. These trials determined that some agents might improve outcome by limiting the extent of brain damage in stroke. Glutamate receptor blockers such as selfotel, cerestat, and eliprodil have failed to show any positive outcome [48]. NMDA-receptor antagonists in animal models have shown some promise when given before or within two hours of ischemic injury. Similar studies with AMPA-receptor antagonists revealed longer therapeutic time windows for intervention with positive outcomes [49]. Both the NMDA- and AMPA-receptor antagonists act by inhibiting cellular depolarization during the ischemic process [2].

Free radical scavengers that target oxygen free radicals have also shown some promise in providing neuroprotection in stroke. Compounds including

ebselen [50] and resveratrol [51] have demonstrated reduction in stroke-related brain damage in animal studies. However other studies involving free radical scavengers including tirilazad and ebselen failed to show any improvement in mortality over a 3-month period [44, 52].

Similarly, modulators of the nitric oxide pathway, such as lubeluzole, showed a neuroprotective effect via down-regulating nitrogen oxide synthase pathway [53]. ICAM-1 facilitates leukocyte migration, which releases cytokines and damages brain tissue. Animal studies have shown that ICAM-1 antibodies (enlimomab, a murine monoclonal antibody against ICAM-1) could reduce infarct volume; however, clinical trials in humans have failed to show similar results [54]. By regulating the antioxidant gene, Nrf2 has a potential as a neuroprotective agent [32, 55]. Further studies have suggested that in addition to pharmacologic neuroprotective agents, other interventions may provide neuroprotection including hypothermia and vagus nerve stimulation (VNS) [56-57].

Therapeutic hypothermia

Even though therapeutic hypothermia showed neuroprotective effects in post-cardiac arrest patients its role in ischemic stroke is controversial. Recent animal studies have shown some benefit in the overall outcome in ischemic stroke patients. Hypothermia can modulate the release of chemical mediators involved in stroke-related neuroinflammation. Mild hypothermia reduces the risk of hemorrhagic conversion and decreases the overall infarct size irrespective of rt-PA administration even though mortality can be higher when hypothermia and rt-PA were given at the same time, within 1-2 hours post MCAO [58]. Some preliminary human data complement animal studies; where supplementing moderate hypothermia to decompressive hemicraniectomy in malignant MCA infarction showed better outcome [59]. However some studies point to the fact that use of hypothermia can interfere with clot lysis effect of thrombolytic agents and decrease the neuroprotective effects [60].

Vagus nerve stimulation

Through its action on norepinephrine and acetylcholine receptors, VNS can theoretically act as an anti-inflammatory modality in central nervous system. In experimental stroke models, brief stimulus of the vagus nerve has been shown to significantly decrease stroke size after focal cerebral ischemia [61-63]. Subsequent to promising results in clinical trials, VNS was approved by FDA for treatment of medically refractory, partial-onset seizures [64] and severe, recurrent unipolar and bipolar depression [65-66]. VNS has been shown to modulate vagus nerve activity, with acute stimulation causing varying degrees of activation or deactivation across the brain in a pulse-dependent manner [57]. Although the effect of these changes is not completely understood, there is strong evidence that vagus nerve activity regulates neurotrophic processes, melanocortin-mediated inflammation, glutamate excitotoxicity, norepinephrine release, and cerebral blood flow [67]. VNS for neuroprotection is a novel field of stroke research with recent studies suggesting a role for the autonomic nervous system, mainly through the beneficial role of parasympathetic tone via the vagus nerve, in the process of cerebral ischemia [56].

Conclusion

Neuroinflammation constitutes a key component in stroke spectrum by participating in the chain reaction occurring during and after ischemia contributing significantly to the brain damage. Anti-inflammation as a neuroprotective mechanism during stroke holds promise in experimental models but currently has shown poor clinical translational power.

Disclosure

No authors report any conflict of interest.

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European Stroke Conference

Time: May 6–9, 2014

Venue: Nice, France

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Deadline for abstract submission: January 12, 2014

The European Stroke Conference (ESC) was founded in 1990 by J. Bogousslavsky (Switzerland) and M.G. Hennerici (Germany). The first meeting was held in Düsseldorf and was attended by about 500 people and proved to be a great success. At that time only the North American conference existed for clinical researchers and basic scientists to present data from stroke research. The prospect to establish another European stroke meeting was highly challenging. After biannual meetings, 1992 in Lausanne and 1994 in Stockholm and increasing attendance, however, the European Stroke Conference became an annual, international, well-received and continuously growing stroke conference. In the meantime this meeting became a highly successful conference with more than 4200 attendees 2013 in London, UK.