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Protective effect of tanshinone IIA, MAPK1, and RELA on ischemic injury after stroke

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Abstract: *Stroke ischemic injury has become one of the major diseases threatening human health and life, and reducing the secondary damage of neurons after cerebral ischemia is an important measure to treat this disease. Inflammatory factors, oxidative stress, and excessive activation of microglia lead to the aggravation of ischemic nerve damage, and if not stopped in time, it will cause large-scale infarction of brain tissue, resulting in permanent neurological deficits. Studies have found that the use of tanshinone II. A can improve the occurrence of the above reactions by inhibiting the two targets of MAPK1 and RELA (p65). This way of inhibiting specific targets to reduce cerebral ischemic damage has become one of the hot issues in today's research. This article mainly reviews the mechanism of tanshinone II. A by inhibiting MAPK and NF-κB signaling pathways, reducing the inflammatory response, oxidative stress, and regulating microglial polarization.*

Keywords: *cerebral ischemia; Tanshinone II.A; MAPK1; RELA(p65)*

1. Introduction

Stroke is the second leading cause of death in the world and one of the leading causes of disability in adults, of which ischemic stroke (IS) caused by cerebral artery occlusion accounts for 85% of all stroke cases, with middle cerebral artery blockage being the most common, and has become one of the major diseases threatening human health and life [1]. IS has a high incidence, recurrence rate, disability rate, and mortality rate, which has caused a serious social burden, which may increase with the continuous increase of China's aging population and the lack of attention to the control of high-risk factors such as hypertension, hyperglycemia, smoking, and blood lipid metabolism disorders [2]. Tissue-type plasminogen activator (t-PA) is a drug approved by the Food and Drug Administration (FDA) for the treatment of cerebral ischemia, but due to its narrow time window (<4.5 hours), there are safety concerns such as bleeding and neurotoxicity risk , and only a small number of patients benefit from the drug. Studies have shown that the inflammatory response after the occurrence is an important mechanism for aggravating secondary brain injury.Therefore, reducing the production and release of pro-inflammatory factors is an effective means of alleviating IS. Mitogen-activated protein kinase 1 (MAPK1) and nuclear factor-κB (NF-κB) p65 are considered to be the two main signaling pathway targets for IS inflammatory regulation [3], and blocking these two targets can greatly improve brain injury.

Danshen is a kind of dried root and rhizome of salvia of the Lamiaceae plant, mainly including more than 30 kinds of lipophilic compounds with tanshinone-type diterpenoid structure and more than 50 kinds of hydrophilic compounds with phenolic acid structure, which has anti-inflammatory, antioxidant, anti-arteriosclerosis, anti-tumor and other pharmacological effects, and is widely used in the treatment of cardiovascular and cerebrovascular diseases [4]. Tanshinone II. A (Tanshinone II.A, TII.A) is a fat-soluble diterpenoid isolated from the dried roots and rhizomes of Danshen [5], which acts on the specific targets of MAPK and NF-κB, the two signaling pathways, MAPK1, and p65, to reduce the inflammation, oxidative stress, and tissue damage caused by the activation of microglia after the

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occurrence of IS, and exert a protective effect on recurrent damage to brain tissue. Clarification of the mechanism of TII.A, MAPK1, and RELA in protecting IS injury is undoubtedly expected to become a new direction for finding more effective treatment measures and complementary therapies.

2. IS injury mechanism

After the occurrence of IS, it can lead to cerebral infarction, edema, neurological dysfunction, vascular destruction, and neutrophil infiltration damage, the main mechanisms include the occurrence of the inflammatory response, an increase of oxygen free radicals (ROS), microglia overactivation, mitochondrial dysfunction, blood-brain barrier destruction and other processes [6], and finally, cause brain dysfunction. Reperfusion is the key to the treatment of ischemic injury, but it can also cause a series of cellular metabolic consequences such as large ROS production, inflammatory response, intracellular calcium overload, and impaired mitochondrial structural function, resulting in ischemia-reperfusion injury [7].

2.1 IS injury and inflammation

In the early stage of the inflammatory response of IS injury, the leukocyte marginal collection adheres to the vascular endothelium, and the function and quantity of integrin CD11/CD18 on the surface of the endothelial cell adhesion molecule-1 (ICAM-1) are highly expressed, and ICAM-1 recruits leukocytes into the brain parenchyma and releases pro-inflammatory mediators to cause an inflammatory response. Neutrophils are the earliest leukocyte subtypes infiltrated after IS, and the release of ROS, metalloproteinase-9 (MMPs-9), and neutrophil elastase are considered to be the main effectors of the blood-brain barrier (BBB) destruction and brain parenchymal nerve cell damage [8]. High-mobility group protein B1 (HMGB1) is a nucleus-bound DNA protein [9], which is involved in the inflammatory response of a variety of diseases, participates in nucleosome production, and regulates gene transcription when IS damage causes cell damage or necrosis, HMGB1 located in the nucleus will be transferred from the nucleus to the cytosol or extracellular, interacting with toll-like receptor 4 (TLR4) and activating TLR4, downward activating the NF-κB signaling pathway, promoting the production of inflammatory factors in cells, aggravating cell and tissue damage.

2.2 IS injury and oxidative stress

In the process of IS, due to ischemia and hypoxia, the mitochondrial oxidative phosphorylation process is interrupted, resulting in mitochondrial depolarization and increased O2-levels, and the acidic environment caused by hypoxia accelerates the conversion of O2- to ROS [10], resulting in mitochondrial swelling and increased permeability, mitochondrial damage is a key factor in the formation of inflammasomes, inflammasome can activate caspase-1 to produce pro-inflammatory cytokines causing brain tissue damage [11], Therefore, the attack of oxidative stress on tissues is always accompanied by the occurrence of an inflammatory cascade. The massive production of ROS can also be activated by degrading the basal layer of collagen and laminin, degrading the extracellular matrix, resulting in increased vascular permeability and BBB destruction, and a significant increase in malondialdehyde (MDA), which represents the degree of oxidative stress damage, and a significant decrease in superoxide dismutase (SOD) activity, which represents antioxidant strength, can be detected after IS [12].

2.3 IS damage and microglia

Microglia are major immune cells in the brain and are key players in neuroinflammation [13], helping to maintain the nervous system in a normal state. Different stages of microtonal cells after IS have different effects, initially manifesting as neuroprotective anti-inflammatory effects and gradually transitioning to a pro-inflammatory phenotype [14]. Studies have shown that the pro-inflammatory factors and myonecrosis factors produced by IS attacks can activate microglia for a long time, and release tumor necrosis factor-a (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and ROS, and these inflammatory factors released by excessive activation of microglia can lead to severe cytotoxicity [15]. The main mechanism that aggravates brain injury after the occurrence of IS is thought to be related to the overproduction of inflammatory factors, oxygen radicals, and microglia [16]. Therefore, reducing the overexpression of inflammatory factors, and antioxidants and regulating the polarization of microglia are important measures for the treatment of re-injury of IS.

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3. The Mechanism by which TIIA inhibits IS damage

3.1. Pharmacological action of TIIA

Given the tissue damage caused by an inflammatory response and oxidative stress caused by IS, the use of some drugs has been shown to combat further damage caused by IS to brain tissue by inhibiting the expression of certain factors, TIIA is one of them, and the detection of the reduction of cerebral infarction area is the criterion. TIIA is the most well-studied and pharmacologically active substance in fat-soluble tanshinone, which is easy to penetrate BBB and can treat or slow down the progression of a variety of diseases, such as Alzheimer's disease, Parkinson's disease, improve myocardial damage, treat pulmonary fibrosis, alleviate liver and kidney diseases, anti-tumor, etc. Studies have found that TIIA has a broad-spectrum neuroprotective effect, exerting anti-inflammatory, and oxidative stress, regulating microglial polarization, and reducing neuronal apoptosis by inhibiting the activation of MAPK and NF-κB signaling pathways, in addition, it can promote vascular generation and promote ischemic recovery Therefore, TIIA Inhibition of the activation of MAPK and NF-κB signaling pathways is considered to be a new target to protect against IS damage.

3.2. Mechanism of TIIA inhibition of MAPK1 and p65

MAPK1 and NF-κB p65 are the targets of MAPK and NF-κB, two typical inflammatory response signaling pathways after IS, and blocking the signal transduction of these two pathways can reduce the recurrent damage of brain tissue. The results show [16] that TII.A treat IS injury by inhibiting these two signaling pathways, and the main effect is to inhibit the NF-κB p65 upstream NIK–IKK conduction pathway, so that the release of NF-κB p65, a representative subunit of downstream heterodimer, is inhibited, and inhibits the phosphorylation of upstream MAPK, so that the phosphorylation level of downstream MAPK1 is inhibited, and the expression level of downstream NF-κB p65 protein is also reduced. It can also downregulate TLR4 and NF-κB high-migration HMGB1 receptors to inhibit the occurrence of the inflammatory response [9]. TII.A inhibits the expression of pro-inflammatory factors TNF-α, IL-6, IL-1β, and ICAM-1 through these two inflammatory pathways, oxidizes stress, increases SOD activity, reduces MDA content, and regulates the transformation of M1 microglia to M2 microglia [16], reducing the degree and extent of IS redamage and protecting brain tissue.

4. Related Roles of the MAPK1 Signaling Pathway in IS

4.1. Overview of the MAPK1 signaling pathway

The MAPK signaling pathway is an important signaling process in which eukaryotic cells mediate extracellular signals and transmit them to intracellular reactions. So far, it has been found that there are 12 types of MAPK, divided into ERK, P38, JNK, and ERK5 four subgroups, these four subfamilies are divided into classical pathway, P38/MAPK pathway, JNK/MAPK pathway, ERK5/MAPK pathway. MAPK1 belongs to the ERK subfamily, MAPK classical pathway, is one of the important members of the MAPK kinase family, MAPK1 gene mRNA expression is closely related to the severity of neurological deficits in IS patients [17], is the main target protein that mediates the pathogenesis of IS. When cells are stimulated by extracellular signals such as inflammatory factors, neurotransmitters, and neurotrophic factors, the MAPK pathway is activated and transmitted to the cell through tertiary signaling. The first stage is the kinase (MEKK or MKKK) of the MAPK kinase is activated, the second stage is MEKK downward activation of the MAPK kinase (MKK), and MKK further activates MAPK as the third stage, through this pathway to regulates cell growth, differentiation, apoptosis, death, and other physiological processes.

4.2. The role of the MAPK1 signaling pathway in IS

MAPK1 has been extensively studied in IS and plays a key role in regulating apoptosis and inflammation. In the early stage of IS, neurons and glial cells are stimulated by inflammatory factors and cytotoxic products, so that the MAPK1 pathway is activated, and the activated MAPK1 will promote the production of inflammatory factors TNF-α, IL-1β, IL-6, and activate microglia, aggravate neurovascular damage and destroy the integrity of the blood-brain barrier, further aggravating the damage [18]. TNF is a typical cytokine involved in the acute phase of systemic inflammation, closely related to the severity of IS, TNF-α can detect its receptor TNF-R1,and TNF-R2 protein expression

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level after the occurrence of IS significantly increased, this protein increase level is regulated by the upstream intracellular MAPK1 signaling pathway, TNF-a binding to its surface receptor, in turn, can promote the activation of MAPK signaling pathway, forming a vicious circle of positive feedback, aggravating the cascade of inflammation. Downregulation of MAPK1 has been shown to reduce TNF-α, IL-1β, and IL-6 levels, and reduce inflammation, oxidative stress, and neuronal damage [19].

5. Role of NF-κB p65 signaling pathway in IS

5.1. Overview of the p65 signaling path and IS

Activation of NF-κB plays an inflammatory role in a variety of diseases such as IS, atherosclerosis, rheumatoid arthritis, asthma, inflammatory bowel disease, and other diseases [20]. NF-κB is a dimeric transcription factor present in mammals, which is involved in the body's inflammatory response, regulating apoptosis and stress response. The NF-κB pathway includes five members: p65, P50, NF-κB2 (P52), RelB, and c-Rel, forming three conduction pathways. NF-κB1 heterodimeric proteins, composed of p65 and p50 [21], play a central role in the inflammatory response. p65, a representative subunit of NF-κB1 heterodimer, is typically phosphorylated at the Ser536 position and is a target for phosphorylation activation by multiple kinases, and its phosphorylation and nuclear translocation are among the most direct and appropriate indicators for assessing NF-κB pathway activation and functional status

The NF-κB signaling pathway is present in the cytoplasm in an inactive form in the presence of the IκB kinase complex (IKK) and prevents NF-κB from entering the nucleus to bind to DNA. When cells are stimulated by various intracellular and extracellular inflammatory factors TNF-a, IL-1β, IL-6, and cytotoxic products, it promotes the activation of proximal cell signaling adaptor protein, activates IKK and phosphorylates and ubiquitinates, resulting in the degradation of IKK protein, releasing p65/P50 heterodimer, which is activated after various translational modifications, so that P65 is isolated from the heterodimer, and then transferred from the cytoplasm to the nucleus and binds to the target gene, causing downstream pro-inflammatory factors TNF-α, IL-6, IL-1β, and iNOS expression [3], macrophage and T cell infiltration, aggravate brain tissue damage. Therefore, it is particularly important to inhibit this conduction pathway to protect the tissue from invasion.

5.2. The p65 conduction pathway regulates microglial polarization and improves IS

When IS damage occurs, various inflammatory factors and cytotoxic products are released, and the NF-κB p65 protein conduction pathway is activated within minutes. Activation of NF-κB stimulates the chemokine CX3CL1 (C-X3-C motif chemokine ligand 1) in neurons, and the enhanced CX3CL1 interacts with its expressed receptor CX3CR1 on microglia to activate microglia [22]. Microglia are activated to polarize [16], releasing various inflammatory factors and cytotoxic mediators that cause tissue damage, causing secondary brain damage. Therefore, inhibiting the binding of P65 to target genes inhibits the excessive activation of microglia through anti-inflammatory and neuroprotective effects, reduces the polarization of microglia to M1 type, and promotes the transformation of M1 type into M2 microglia with neuroprotective effect, which is considered to be another therapeutic strategy to effectively treat secondary damage caused by IS [23].

6. Relationship between MAPK1 and NF-κBp65 inflammatory conduction pathway

MAPK1 is the integration point of a variety of biochemical signaling pathways, NF-κB is one of the key transcription factors of the MAPK signaling pathway, its activation is essential for the expression of multiple genes such as inflammation and cell proliferation, and inhibition of MAPK1 activation can subsequently inhibit the inflammatory response caused by NF-κB. Early in the occurrence of IS injury, the activation of the MAPK1 protein phosphorylation pathway in neurons and glial cells can mediate the nuclear translocation caused by NF-κBp65 [2], and activated NF-κBp65 can cause a series of inflammatory responses, with the activity peaking ten minutes after activation. Although the MAPK signaling pathway is activated for a long time, the activation of the NF-κBp65 signaling pathway has strict time control factors because NF-κBp65 needs its upstream signaling factor IKK to be activated by phosphorylation, generalization, and other modifications when it is activated, and it needs to be newly synthesized[24]. When MAPK1 phosphorylation is inhibited, cascade activation of NF-κBp65 is also inhibited, and a significant decrease in NF-κBp65 protein expression can be detected. Therefore, the

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use of TIIA can not only directly inhibit the activation of NF-κBp65 signaling pathway, but also inhibit the activation of NF-κBp65 downstream through MAPK1/MAPK signaling pathway, reduce inflammatory response, oxidative stress, etc.

7. Conclusion and outlook

In summary, IS is a disease with complex etiology and multiple factors that jointly restrict its occurrence and development. A variety of substances produced by IS damage can activate MAPK, NF-κB signaling pathway, there is the interaction between these two signaling pathways, and eventually produce a cascade reaction to aggravate the damage of IS, and each step of this cascade reaction has a potential intervention pathway, effectively blocking this cascade reaction process, which is the focus of current drug treatment. TIIA is a compound with the specific targets of inhibiting MAPK and NF-κB, which play a key role in improving the inflammatory response, oxidative stress, and microglial polarization caused by IS through this interactive pathway, and under the combined action of these processes, the damage such as cerebral infarction area, edema degree, and neurological dysfunction is reduced.Based on the signaling pathway activated when IS occurs, it is necessary to study the target of drugs at a deeper level and find drugs that inhibit the damaging signaling pathway, which provides a new basis and reference for the treatment and prognosis of IS injury, plays a neuroprotective role, promotes the selection of therapeutic targets, and provides new ideas and research directions for reducing IS injury. Studies have shown the protective effect of TII. A on cerebrovascular diseases has been affirmed, and it has been clinically used in the treatment of the nervous system, circulatory system, and other diseases, with broad clinical application prospects, but for the long-term prognostic effect and specific pharmacological mechanism of the disease, scholars still need to do further research and bring breakthroughs to the treatment of diseases.

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