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Review Article
Role of NF-κB on Neurons after Cerebral Ischemia Reperfusion

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Abstract

The transcription factor NF-κB which is involved in inflammation and cell survival is a critical regulator of hundreds of genes. It has been demonstrated that there is a connection between NF-κB and cerebral ischemia reperfusion. The activation of NF-κB in ischemia can be triggered by reactive oxygen species and several inflammatory mediators and is closely related to the release of IκB. Moreover, the NF-κB subunits of RelA and p50 are mainly responsible for the pernicious effect after cerebral ischemia reperfusion. Recently, the role of NF-κB on neurons has been widely investigated and the major studies have shed light on the pro- and anti-apoptotic function for NF-κB. Whether NF-κB promotes or inhibits apoptosis appears to depend on the stimulus, specific cell type and duration. Here, the available research for the role of NF-κB on neurons after cerebral ischemia reperfusion is reviewed.

Key words: NF-κB, cerebral ischemia reperfusion injury, neurons, apoptosis, neuroprotective effect

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INTRODUCTION

Ischemic stroke is one of the leading causes of mortality and disability in the world. Ischemia, which is often followed by reperfusion, can elicit further problems including inflammatory response, apoptosis and neuronal damage[1-3]. Cerebral ischemia reperfusion injury (CIRI) refers to the recovery of blood after a certain period of cerebral ischemia fails to recover its function and aggravates brain dysfunction[4]. CIRI poses a serious risk to human health and causes serious social and economic problems, which place a huge burden on families and society. Because of its etiology and complex pathogenesis, it has drawn more and more investigators' attention in the medical research[5,6]. Therefore, it is of great clinical significance to explore and study the mechanism and treatment of CIRI.

The transcription factor NF-κB, which is involved in cell survival, cytokine production and inflammation, is a key regulator of hundreds of genes. Since it has been reported in 1986[7] as a B-cell nuclear factor, NF-κB has become the focus on the study of cerebral ischemia. A large number of animal experiments have shown that the expression of NF-κB changes if cerebral ischemia is taken place. This study reviewed the mechanism of NF-κB on neurons after cerebral ischemia reperfusion.

Cerebral ischemia reperfusion injury: Cerebral ischemia-reperfusion injury is further a complex pathological process of brain tissue caused by cerebral ischemia-reperfusion[8,9]. The mechanism of CIRI has undergone a continuous deepening process from organ level to cell level and molecular level[10]. Ischemia-reperfusion injury, which is resulted in cerebral infarction and edema, is the main cause for the aggravation of cerebral injury and functional impairment. Experimental studies have firmly established that cytotoxic effect of excitatory amino acids, oxidative stress, overload of calcium inside neurocytes, cascade inflammatory reactions and apoptosis are the main reasons for the CIRI[11-13]. These factors interrelate with each other and further promote the destruction of neurological function and the formation of cerebral infarction.

Excitatory amino acids: Excitatory amino acids, including glutamate (Glu) and aspartic acid (Asp), are the predominant excitatory neurotransmitter in the brain, which play a crucial role in the information transmission. The importance of Glu in normal synaptic function has been well described[14]. The amounts of Glu in the synaptic cleft that can lead to neurotoxicity have been established. In the central nervous system (CNS), Glu, an important factor following CIRI, may induce an increase in intracellular calcium and the generation of free radicals, culminating in cell death, necrosis or apoptosis[15].

The neuronal excitotoxicity event begins with the activation of the ionotropic receptor of glutamate called α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) by a large number of glutamate and then activates the phosphatidylinositol signal transduction pathway coupled to G-protein[16]. The process leads to the change of cell permeability. It can trigger the entry of extracellular excessive amounts of sodium and chloride ions, together with water, into the cell. Eventually, it causes cytotoxic brain edema that leads to cell death, necrosis or apoptosis.

Oxidative stress: It has been extensively studied that oxidative stress and excessive formation of free radicals are implicated in mitochondria dysfunction after cerebral ischemia-reperfusion[17,18]. Oxidative stress depolarizes mitochondrial membrane potential by lipid peroxidation, which further leads to mitochondrial dysfunction[19]. Mitochondrial dysfunctions occur as a consequence of cerebral ischemia and promote ischemia-induced neuronal cell death, especially the intrinsic pathway of apoptotic cell death[20,21]. In addition, prolonged ischemia reperfusion returned to aggravate mitochondrial dysfunctions caused by oxidative stress. Mitochondria plays a role in the generation of reactive oxygen species (ROS) and regulation of apoptosis, as important factors in the pathogenesis of neurodegenerative diseases and cerebral ischemia[20,22]. It was reported that transient cerebral ischemia, followed by reperfusion, significantly increased the generation of ROS, nitric oxide (NO) and lipid peroxidation end-products, as well as markedly reducing levels of the endogenous antioxidant glutathione[23]. Prolongation of reperfusion was shown to increase the level of MDA, which ascribed to lipid peroxidation end-products. This suggested that ischemia reperfusion is associated with oxidative stress damage of mitochondria in the peri-infarct area[24].

Inflammation: Recent studies have confirmed the pivotal role of inflammatory response in the pathogenesis of acute ischemic stroke[25]. The occurrence of cerebral ischemia is characterized by infiltration of various types of inflammatory cells (mainly leukocytes, microglia and astrocytes). It can trigger the subsequently production of inflammatory factors including cytokines (IL-1 IL-6 TNF-α), chemokines

(MCP-1, MIP-1α) and adhesion molecules, which play an important regulatory function in ischemia reperfusion. Experimental data have demonstrated that resident microglia are activated within minutes of ischemia onset and produce a plethora of proinflammatory mediators, including IL-1 and TNF-α, which exacerbate tissue damage[26,3]. The IL-6 is involved in the process of ischemia reperfusion by participating in the immune response and inflammatory response. Its expression level can be regarded as an important indicator to determine the ischemia reperfusion injury. Meanwhile, TNF-α and IL-1β participate ischemia reperfusion injury to produce a variety of tissue factors with the release of EAA, NO and oxygen free radicals by promoting the occurrence of inflammatory response and activating endothelial cells.

Calcium overload: Earlier study had shown that total brain calcium content rises during reperfusion following prolonged cerebral ischemia[27,28]. With the onset of ischemia, neurons and glia deprived of oxygen and glucose lose energy stores, become depolarized and release large quantities of glutamate into the extracellular space. The resulting overstimulation of neuronal glutamate receptors, conspicuously N-methyl-D-aspartate (NMDA) receptors, leads to leakage of intracellular potassium and excessive influx of calcium through receptor-gated ion channels and metabolic derangements[29]. Calcium overload and a series of harmful metabolic reactions triggered serve as the key that results in neurons injury and death. The process takes place through mitochondria dysfunction, activation of phospholipases and production of cytotoxic substances after CIRI[30,31].

Cell apoptosis: Apoptosis refers to the process of cells demise under certain physiological or pathological conditions, in which the cell is controlled by the internal genetic mechanism. Apoptosis, the main pathway that leads to the death of delayed neuronal cells[32], is a critical pathological process in cerebral ischemia-reperfusion. It has been demonstrated that caspase family is the key factor and execution in the processes of apoptosis. Huang Xiaoping *et al.*[33] showed significant increase of caspase protein and neuronal apoptosis following by the onset of cerebral ischemia reperfusion. The process of apoptosis is regulated by gene including bcl-2 and bax. The bcl-2 has the function of ameliorating apoptosis and death caused by brain injury and bax can promotes apoptosis by inducing the release of cytochrome C. Experimental studies have showed that increase of antiapoptotic Bcl-2 protein in the mitochondria could inhibit translocation of Bax and release of cytochrome C[34]. It can significantly decrease the activation of caspase thus to inhibit apoptosis. The regulation of apoptosis depends on the ratio of bcl-2/bax. Qiu *et al.*[35] studies showed that improvement of the ratio of bc1-2/bax by increasing expression of bcl1-2 and reducing expression of bax protein can play an anti-apoptotic role, which has a neuroprotective effect.

NF-κB: NF-κB is an important nuclear transcription factor, which can transfer information from cytoplasm to nucleus. It triggers the expression of corresponding genes encoding acute-phase proteins, cell adhesion molecules, cell surface receptors and cytokines[36]. It has been well established that NF-κB, mainly distributed in nerve cells, astrocytes and microglia, is a key regulator involved in cell survival, inflammation and immunization. The NF-κB is a member of Rel protein family that consists of p50, p52, RelA (p65), RelB and C-Rel. They share a N-terminal 300 amino acid Rel-homology domain (RHD), which allows dimerization, nuclear translocation and DNA binding[37]. Within the CNS, the p50/RelA heterodimer is mostly the transcriptionally active form of NF-κB[38,39].

Activation of NF-κB: It has been found that a variety of factors could induce the activation of NF-κB such as: (1) Exogenous factors, including ultraviolet, ionizing radiation, microbial pathogens and chemotherapeutic drugs. (2) Endogenous factors, including cytokines (TNF-α, IL-1, lymphotoxin), inflammatory mediators (platelet activating factor, leukotriene, reactive oxygen species), growth factors (IL-2, macrophage colony stimulating factor, platelet derived growth factor), immune related receptors (CD3, CD18, TCR, etc.) and oxidative stress. The activation signal of NF-κB in the CNS can be divided into two categories, the first of which is similar to aforementioned and the other is specific signal such as depolarization, neurotransmitters, nerve growth factors and some neurotoxin stimulation[40].

When the cells are in resting state, NF-κB are presented in the cytoplasm binding its inhibitory protein (I κB) into a trimer in an inactive form. In this way, NF-κB does not have the function of gene regulation and transcription. However, it can be mobilized by either the classical or alternative pathway[41]. The classical pathway is thought to be related closely with the release of IκB. When stimulated by activation signal such as TNF-α, IκB kinase (IκK) overexpressed and phosphorylated serine residues 32 and 36 of IκK-α. Subsequently, it leads to their ubiquitination and degradation through the proteasome pathway[42], so that NF-κB can rapidly transfer into the nucleus and combine with the DNA-specific sequence. Finally, it induces the transcription and higher expression of target gene[43,44]. The alternative pathway, in addition, involved an

important component called NF-κB-inducing kinase(NIK) which actives IκK-α. Furthermore, it has been demonstrated that NF-κB can regulate transcriptional activation via IκBs dissociation induced by tyrosine phosphorylation. Then, NF-κB structural changes p65 caused by p65 subunit phosphorylation and IκK independent activation[45].

NF-κB in cerebral ischemia reperfusion: As a pleiotropic transcriptional regulator protein, NF-κB involved in a variety of biological processes such as immune and inflammatory response, apoptosis and so forth[46]. It has been found that NF-κB can regulate the expression of cytokines, inflammatory mediators and cell adhesion molecules through binding with kappa-B site resided in the target gene enhancer[47]. There is ample evidence that NF-κB becomes activated after onset of cerebral ischemia reperfusion, the activation of NF-κB then initiates the transcription of relevant target gene[48-50]. It participates in the pathophysiologic process by promoting inflammatory response, mediating free radical damage and apoptosis. Moreover, these pathways are not isolated, but promote and interact with each other mutually.

Stephenson *et al.*[48] demonstrated the activation of NF-κB by detecting the expression and transactivation of NF-κB in nuclei of neurons with immunohistochemistry and electrophoretic mobility gel-shift analysis at adult rats. The rats were subjected to 2 h of focal ischemia induced by middle cerebral artery occlusion (MCAO)and 2, 6 and 12 h after reperfusion. Feng *et al.*[51] revealed significantly increased expression of NF-κB in cerebral ischemia reperfusion by determine the translocation from cytoplasm to nucleus of NF-κB in the ischemic hemisphere versus normal, sham operation and nonischemic hemisphere. It further confirms the activation of NF-κB in cerebral ischemia reperfusion.

Role of NF-κB on neurons after cerebral ischemia reperfusion: Accumulation of data has revealed that NF-κB activation could be either protective or deleterious. In cerebral ischemia-reperfusion, the transient activation of NF-κB can induce the expression of neuronal protective factor in hippocampus and protect the brain tissue. Nevertheless, the sustained or overexpression of NF-κB can lead to neurons death in hippocampus. Therefore, it can be concluded that NF-κB serves a dual function as a regulator of neurons survival in pathological conditions. It is considered that the effect of NF-κB on brain mainly depends on the region, stimulus, dose and duration specific[52]. Chen *et al.*[53] found that the type and the amount of NF-κB subunits play a decisive role in apoptosis. It appeared that overexpression of RelA inhibits apoptosis, whereas overexpression of c-Rel enhances apoptosis. It can be concluded that NF-κB is closely interrelated with apoptosis. Furthermore it is involved in the transcriptional regulation of a variety of apoptosis-related genes, which have a bi-directional effect of inhibiting and promoting apoptosis[54].

Neurotoxic effects of NF-κB: Much work of NF-κB family proteins has proposed that the NF-κB is activated after cerebral ischemia and excessive expression of NF-κB is detrimental to neurons survival[55]. Nurmi *et al.*[56] confirmed that activated NF-κB is induced and translocated to nuclei of neurons after stroke by observing the expression of NF-κB in the cytoplasm of neurons after death of 23, 28 and 38 h in human stroke. Previous study has confirmed that neurotoxic effects of NF-κB are associated with factors containing inflammation, free radical and pro-apoptosis[18,41,44].

NF-κB with inflammation: The NF-κB is an important signal transduction molecule involved in the inflammatory response after cerebral ischemia-reperfusion. It is an intermediate hub of intracellular signal transduction pathway, which is stimulated by activation signal and binds to DNA-specific sequence. Then, it induces transcription and expression of related genes to activate proinflammatory factors, leading to the occurrence of inflammatory responses after ischemia and ultimately aggravating the cerebral disease[57]. So far, the role of inflammatory cytokines in CIRI has received the considerable attention[58]. The NF-κB is considered to be the initiating factor in the inflammatory cascade reaction after cerebral ischemia[58] and is involved in the development and progression of CIRI. As an important transcription factor, NF-κB plays a key role in the transcription of inflammatory response genes, including adhesion molecules, cytokines and so on Crack and Wong[18]. Under normal physiological conditions, NF-κB usually has a low level of activity in neurons, glial cells and cerebrovascular endothelial cells. In cerebral ischemia, it can be increasingly activated and promotes the expression of cytokines subsuming IL-1, IL-6, IL-8 and TNF-α[49,59,60]. Whereas, these cytokines can activate NF-κB in turn so that the activation of NF-κB further proliferates, which finally exacerbates the inflammatory response[60]. Ueno *et al.*[49] confirmed that inhibiting the expression of TNF-α, IL-1β and cell adhesion molecule mRNA significantly attenuated the neuronal damage 7 days after global brain ischemia. Berti *et al.*[61] examined mRNA expression levels for cell adhesion molecules and inflammatory cytokines at five time points (3, 6, 12, 24 and 72 h) after transient middle cerebral artery occlusion (MCAO). At all time points examined, activated NF-κB immunoreactivity was observed in cells throughout the infarct-damaged tissue, which suggests that NF-κB-mediated inflammatory processes are associated with CIRI.

NF-κB with free radical: Oxygen free radicals have been widely implicated in the pathogenesis of brain injury due to ischemia followed by reperfusion. After the onset of ischemia, a large number of oxygen radicals containing superoxide anion and hydroxyl radicals are generated. It causes oxidative damage and triggers chain reaction, which results in production of a variety of reactive oxygen species (ROS). Cerebral injury causes lipid peroxidation decreased membrane fluidity and higher permeability, protein denaturation and nucleic acid peroxidation by ROS. All this can be attributed to the activation of NF-κB. In addition, ROS is involved in nerve injury through NF-κB pathway[62,63]. Alternatively, the activation of NF-κB can promote the expression of free radical, which in turn activates NF-κB. As a result, it leads to create a positive feedback loop that amplifies the reaction.

NF-κB with pro-apoptosis: Studies have shown that apoptosis is involved in the formation and progression of neurotoxicity after CIRI[64,65]. After cerebral ischemia, NF-κB was specifically activated and caused neuronal damage or even death by regulating the expression of a variety of apoptosis-related genes[43]. The NF-κB is widely activated in neurons and glial cells after brain injury, which subsequently induced the production of pro-apoptotic genes, proteins (such as COX-2, iNOS, P53 etc.) and cascade reaction. Ultimately, the activated caspase leads to neuronal apoptosis[66]. Desa *et al*.[62] found that the application of NF-κB specific inhibition can block the expression of inflammatory factors. Moreover, it significantly ameliorates the cerebral ischemia-induced neurological deficits, which suggests a neuroprotective effect.

Neuroprotective effect of NF-κB: Whereas, NF-κB is commonly associated with cell damage and apoptosis under different pathological conditions, it has been proved that the activation of NF-κB has protective effects on neurons. The neuroprotective effect of NF-κB is mainly through anti-apoptosis.

Anti-apoptosis of NF-κB through anti-apoptotic proteins: The activation of NF-κB can mediate the cytoprotection reaction and induce the expression of anti-apoptotic proteins such as Bcl-2, Bcl-x and calcium-binding protein to prevent apoptosis. Therefore, it can protect neurons and reduce cell death by resisting oxidative and metabolic damage. Beg *et al*.[67] firstly confirmed the anti-apoptosis function of NF-κB. Their experiments found that Re1A-null rats died of a massive degeneration of liver. Researches in previous years provided that anti-apoptosis proteins such as IAPs, Bcl-2 family and TNFR-associated factors are involved in the process of anti-apoptosis after activation of NF-κB[43,68,69]. Moreover, NF-κB can play an anti-apoptotic role by inhibiting self-phagocytosis[70]. The NF-κB is involved in cells survival associated with cell viability and growth factors. It was well documented that transforming growth factor-β has anti-apoptotic effects in cerebral ischemia and neuronal culture, which increase transcriptional activation of NF-κB in a time-concentration-dependent manner[71]. Irving *et al*.[50] detected the activation of NF-κB in the middle cerebral artery 3 h after occlusion. The nuclear translocation and binding activity decreased from 6-8 h after occlusion, which suggests the decreasing activation of NF-κB may exacerbate ischemic-induced cell death.

Anti-apoptosis of NF-κB through signaling pathway: The anti-apoptosis of NF-κB is a complex series of process involving multiple signaling pathways. It is mainly achieved by inducing or up-regulating the expression of anti-apoptotic genes. These genes have NF-κB binding sites located in regulatory sites and their expression products play a neuroprotective effect by inhibiting cell death receptors or mitochondrial damage. Because of interdigitating signaling and feedback pathways, NF-κB plays an important role in several signal pathways after cerebral ischemia-reperfusion.

Mitogen-activated protein kinases (MAPKs), a group of serine/threonine protein kinases, comprising three well-characterized subfamilies: Extracellular regulated kinases 1 and 2 (ERK1/2), the c-Jun N-terminal kinases (JNKs), which are also known as stress-activated protein kinases and the p38 MAPKs[72,73]. There are strong evidences that all three MAPK signaling pathways have been implicated in NF-κB activation through the phosphorylation of its inhibitor termed I-κB, which is correlated with the regulation of apoptosis after cerebral ischemia-reperfusion.

It has been well known that the c-Jun N-terminal kinases (JNK) signaling pathway acts on the regulation of apoptosis and is expected to be an important target for the treatment of apoptosis-related diseases. In addition, it has been shown to be a potential cascade mediating neuronal apoptosis triggered by ischemia[74]. Studies have demonstrated that NF-κB is involved in the JNK after ischemia as ROS are potent inducers of JNK10. Javelaud and BesancEon[75] provided evidence that the repression of JNK activation by NF-κB participates in the anti-apoptotic effect. Additionally, it has been proved that the extracellular signal regulated kinase 1 and 2 (ERK1/2) pathway contributes to the neuroprotective effect against oxidative damage in hippocampal neurons[76].

CONCLUSION

Studies have discovered the momentous role of the transcription factor NF-κB in cerebral ischemia that can be beneficial for the clinical therapy. In certain situations, NF-κB acts as an anti-apoptotic protein, whereas in others it mainly functions as pro-apoptotic transcription factor that contributes to acute neurodegeneration. Also, many details of how NF-κB acts in cerebral ischemia reperfusion that lead to the neurologic damage warrant further investigation. However, on the basis of present knowledge, it is already obvious that NF-κB offers many targets for therapeutic intervention in neurodegeneration after cerebral ischemia reperfusion.

SIGNIFICANT STATEMENT

This study discovered the momentous role of the transcription factor NF-κB in cerebral ischemia that can be beneficial for the discovery of therapeutic targets for CIRI. This study will help the researchers to uncover the critical areas of factors affecting cerebral ischemia that many researchers were not able to explore. Thus a new theory on the role of NF-κB on neurons after cerebral ischemia reperfusion may be arrived at.

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