Potential Mechanism and Pathways in Cerebral Ischemia–Reperfusion Injury: Therapeutic GLANCE

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Abstract

Cerebral ischemia-reperfusion injury is a progressive disease that results in the lack of oxygen and nutrients needed for cellular metabolism in neurons due to blood flow disorders. The pathogenesis of this disease is different; however, it has been shown that the onset of inflammation interacts with I/R through the production of active oxygen species and increases the apoptosis of the neural cells. Therefore, different signaling pathways interfere with the induction of inflammation and the production of active oxygen species. Therefore, the common point of these pathways leads to the appearance of apoptosis-inducing molecules and inhibit the expression of anti-apoptosis molecules such as BCL-2. In the other hand, due to the dual role of some of these pathways in apoptosis and angiogenesis, it can be said that further studies can be useful in finding suitable therapeutic strategies based on the pathogenesis of Cerebral ischemia-reperfusion injury inducing angiogenesis in order to repair damaged veins and prevent disease progression.

Keywords: Reperfusion; Nitric Oxide; Reactive Oxygen Species; Therapeutics

INTRODUCTION

Cerebral ischemia-reperfusion injury (I/R) is a lethal disease, with increased prevalence in the world (1). This condition occurs due to cerebrovascular ischemia as a result of impaired blood flow. Risk factors such as thrombosis or bleeding caused by blood vessels destruction increase the risk of I/R. This disorder reduces the oxygen and other materials needed for neurons in the brain tissue, which ultimately leads to apoptosis and death of neurons (2). One of the main strategies for treating ischemic brain tissue is to restore the blood flow to the brain. However, restoring blood flow leads to the initiation of a series of pathogenic mechanisms such as increased intracellular calcium, excessive production of reactive oxygen species (ROS) through endoplasmic reticulum (ER) and mitochondrial dysfunction, and ultimately inflammatory responses increase as a result of immune system cells...
activation. Hence, identification of pathways leading to apoptosis and death of neuronal cells by restoring blood flow to the brain tissue can present a supportive treatment that increase survival. In this review we discuss pathogenic mechanisms followed by the recovery of blood flow in patients with I/R injury and the strategies to decrease I/R.

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Attenuation of Nitric Oxide Production

Nitric oxide (NO) is a factor that is produced by several cells such as endothelial cells (ECs). NO plays several roles in homeostasis and the function of neural cells due to the regulation of neural synapses displacement, and the flexibility of the blood vessels wall. However, studies have shown that excessive NO production after I/R can interfere with the function of brain neurons and ultimately leads to apoptosis. The results of these studies have shown that caveolin-1 (Cav-1) inhibits the production of NO in normal conditions in the blood vessels. Cav-1 is one of the scaffolding proteins on the ECs membrane, which plays an important role in the permeability and displacement of materials in blood vessels. On the other hand, Cav-1 is characterized to increase angiogenesis through NO and vascular endothelial growth factor (VEGF) production. However, after the I/R, NO production increase by ECs as a result of Cav-1 inhibition and ultimately NO stimulate Matrix metalloproteinase (MMPs) and increase the permeability of the blood-brain barrier and increase the production of proinflammatory chemokines and cause inflammation which leads to neuronal cells apoptosis. Studies showed that some tight junctions, such as VE-cadherines, are involved in ECs integrity but the expression of MMPs as a result of degradation and dysfunction of the tight junctions in ECs increases the permeability of the blood-brain barrier and increase the production of proinflammatory chemokines and cause inflammation which leads to neuronal cells apoptosis. Studies showed that some tight junctions, such as VE-cadherines, are involved in ECs integrity but the expression of MMPs as a result of degradation and dysfunction of the tight junctions in ECs increases the permeability of the blood-brain barrier and disrupts the exchange of the materials. In the case of oxidative stress, AMP-activated protein kinase (AMPK) inhibits Cav-1, however studies showed AMPK induces angiogenesis due to NO production. In t other hand, studies have shown that AMPK pathways regulate VE-cadherines in ECs via regulating MMPs expression. Therefore, considering the dual role of the AMPK pathway in regulating the expression of MMPs, and inducing angiogenesis is noticeable, so identifying the pathways regulates MMPs and induces angiogenesis through AMPK can be an appropriate therapeutic strategy to prevent I/R. It also prevents neuronal cells apoptosis through inhibiting caspase 3. Identifying pathways that increase angiogenesis via AMPK pathway through NO production can be a suitable strategy for inducing angiogenesis and repairing the damaged veins after I/R. In the other hand, the increased expression of NO-inflammatory mediators has been shown to activate P38 / MAPK pathway and ultimately impairs the function of the neuronal cells. Treating I/R patients with ferulic acid which activates p38 mitogen-activated protein kinases (P38/MAPK) pathway and increase BCL-2 expression which prevents neuronal cells apoptosis. P38 / MAPK increase HMGB1 expression. HMGB1 interact with TLR4 at the surface of neutrophils and increase NO production and activates NF-KB pathway which increase inflammatory response. The dual role of the P38 / MAPK pathway identifies the pathways which produce apoptotic and antiapoptotic factors can provide an appropriate therapeutic strategy to prevent neuronal cells apoptosis due to overproduction of NO. However excessive NO production plays an important role in the pathogenesis of I/R, the identification of pathways which induce angiogenesis through NO can control the damage which occur as a result of NO overproduction and ultimately prevent the expansion of I/R.

Reactive oxygen species

It seems that ROS production is particularly important among pathogenic factors that lead to the destruction of neurons after I/R, because the treatment of these process is very difficult. NOX2/4 is one of the main factors in the production of ROS in brain tissues ,and various factors affect the expression of NOX2/4 in I/R. Tumor growth factor-β (TGF-β) interactions with its receptor activates SMAD2/3 pathway and activating receptor-like kinase 5 (ALK5) increase the expression of NOX2/4. LOX-1 is another factor which increases the expression of NOX2/4. Studies showed that increased expression of LOX-1 in ECs in brain tissue is accompanied by an increase in the expression of NOX2/4. Nuclear factor erythroid 2-related factor 2 (Nrf2) is one of the main factors controlling the production of ROS, which acts as an antioxidant agent. The results of recent studies showed that the lack of expression of
Nrf2 is accompanied by an increase in the expression of LOX-1. Therefore, the expression of Nrf2 in I/R decreases\(^{26,27}\). Also, expression of Nrf2 is regulated by casein kinase 2 (CK2), so the reduction of CK2 expression is associated with the decrease of Nrf2 expression \(^{28,29}\). The phosphatidylinositol 3 kinase (PI3K/AKT) pathway is in the up-stream of Nrf2 \(^{30}\). Studies showed that phosphorylation of PI3K / AKT activate mTOR, which ultimately increases the expression of Nrf2. The interaction of Rac-1 with BCL-2 leads to impairment in mitochondrial function and increased ROS production. The intersection leads to the control of the PI3K/AKT / mTOR pathway and ultimately increases the expression of NOX2 \(^{31}\). Rac-1 increase the release of cytochrome c (cyt. c), as well as an increase in the expression of fas-L, which ultimately lead to neuronal cells apoptosis due to increased production of ROS through activating by activating MLK3 / JNK / c-JUN pathway \(^{32}\).

**Inflammatory mechanisms & pathways**

According to experimental and clinical evidence, inflammation plays important role in the development of I/R. Studies showed that the regulation of inflammatory responses is regulated by the expression of pro and pre inflammatory cytokines \(^{33}\). However, recent studies have shown that imbalance in the expression of pre-inflammatory and proinflammatory cytokines is impaired after I/R \(^{34}\). Peroxidase proliferator-activated receptor gamma (PPARγ) is one of the factors that normally has anti-inflammatory function. PPARγ expression decreases after I/R. This factor reduce inflammation through reducing the production of inflammatory cytokines such as (TNF-α) IL-1, IL-6 and Tumor necrosis factor-α and also by regulating the expression of MMPs and its inhibitors \(^{35}\). Additionally JAK / ERK / STAT pathway activation after I/R increases the production of inflammatory cytokines. Studies showed this pathway increases immune responses and inflammation due to increased expression of MMPs and inflammatory cytokines. On the other hand, considering to this fact : PPARγ reduces inflammation by modulating MMPs and their inhibitors, we can hypothesize that probably (PPARγ) can reduces inflammation by inhibiting the JAK / ERK / STAT \(^{35,36}\). Several studies showed that PPARγ increases the expression of hypoxia inducible factor 1 (HIF-1). HIF-1 reduces the inflammation by reducing the production of IFN-γ and increasing the production of IL-4 and IL-10 cytokines that suppress inflammatory responses. However, the expression of HIF-1 is also reduced after I/R \(^{37,38}\). PPARγ reduces inflammatory responses via controlling the nuclear factor kappa-light-chain-enhancer of activated B cells too. NF-kB increases the incidence of inflammation by increasing the expression of sticky molecules such as ICAM-1 and VCAM-1, as well as the production of inflammatory cytokines. One of the main factors behind the NF-KB is the Toll-like receptor (TLR), several studies showed that the expression of TLR has increased after I/R activates NK-KB pathway and increased the inflammation \(^{39,40}\). Studies showed that increase in the expression of ICAM-1 due to inflammation as a result of NF-kb activation increase the pro-apoptotic molecules and eventually destructs EC cells and impaired cerebral blood barrier function. Consequently, increasing the expression of ICAM-1 after I/R may be a predictor of brain dysfunction and disease progression \(^{41}\).

**Strategies for cerebral ischemia–reperfusion injury treatment**

Several strategies has been designed and implemented to prevent I/R but there is no complete treatment for patients. Disturbance in the molecular mechanisms between the brain cells such as microglial and astrocytes can be the main reason of the disease, most of the recent therapeutic approaches target the molecular pathways to improve the function of the brain cells \(^{42}\). We mentioned several drugs and their mechanisms in table 1. Most of the recent therapeutic approaches have led to therapeutic treatment of molecular pathways to improve the function of the brain cells. For example, studies have shown that brahma-related gene 1 (Brg1) is one of the regulators in the cell nucleus, which plays an important role in regulating the expression of transcription factors such as Nrf2. Studies have shown that increasing the expression of Brg1 after I/R can increase the expression of Nrf2 through reduce the intracellular ROS and prevents apoptosis \(^{43}\). Higenamine can increase the expression of Nrf2 and prevent the progression of I/R through regulating the PI3k/AKT pathway. Inflammatory responses and inflammatory mediators such as IL-1 and TNF-α can lead to I/R. Studies have shown that impaired NF-kB pathway
plays an essential role in inflammation so berberine can regulate NF-kB pathway and prevent inflammation and I/R. Plumbagin is a drug which prevents inflammation and can be used in I/R treatment. Studies have shown that Plumbagin inhibits NF-kB pathway and prevents the production of inflammatory cytokines and MMPs. There are several medications used to treat I/R. It has been shown that the focus of treatment design is on inflammatory pathways and ROS production because preventing apoptosis decrease I/R and increase the patients’ survival.

**CONCLUSION**

Finally, it can be concluded that due to the different signaling pathways PPARγ can prevent inflammation, the expression of PPARγ is reduced after the I/R so we can hypothesize that identifying the pathways that enhance PPARγ expression can be used as a target therapy in I/R treatment. NF-kB and PI3K/AKT are common pathways in inflammation and ROS production, and activation of PI3K/AKT and inhibition of NF-kB can significantly reduce the incidence of apoptosis in ECs and neurons. Therefore future therapeutic approaches can be directed toward these pathways, and targeting these two pathways will increase patient survival and prevents the I/R.

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**COMPLIANCE WITH ETHICAL STANDARDS**

Conflict of interest

The authors declare no conflict of interest.

Research involving human participants and/or animals

This article does not contain any studies with human participants or animals performed by any of the authors.

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