Behavioural Brain Research 336 (2018) 32–39

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Nrf2 inhibits NLRP3 inflammasome activation through regulating Trx1/TXNIP complex in cerebral ischemia reperfusion injury

Yanghao Hou[a,1], Yueting Wang[a,1], Qi He[a], Lingyu Li[b], Hui Xie[a], Yong Zhao[b], Jing Zhao[a,*]

[a] Department of Pathophysiology, Chongqing Medical University, Chongqing, People's Republic of China
[b] Department of Pathology, Chongqing Medical University, Chongqing, People's Republic of China

ARTICLE INFO

Keywords:
Nuclear factor erythroid 2-related factor 2
Nod-like receptor protein 3 inflammasome
Thioredoxin1
Thioredoxin interacting protein
Inflammation

ABSTRACT

The nod-like receptor protein 3 (NLRP3) inflammasome has a critical role in inflammation damage in ischemic injury, and the activation of the inflammasome is closely related to the interaction with thioredoxin interacting protein (TXNIP), which dissociates from the thioredoxin1 (Trx1)/TXNIP complex under oxidative stress. However, the negative regulator of NLRP3 inflammasome activation has not been fully investigated. Nuclear factor erythroid 2-related factor 2 (Nrf2) takes on a critical part in the antioxidant stress system, that controls the driven genes of antioxidant response element (ARE). Activate Nrf2 could inhibit the activation of NLRP3 inflammasome in acute liver injury and severe lupus nephritis. We aimed to explore the protective effect of Nrf2 in inhibiting the NLPR3 inflammasome formulation through the Trx1/TXNIP complex in cerebral ischemia reperfusion (cerebral I/R) injury. Middle cerebral artery occlusion/reperfusion (MCAO/R) model was used to imitate ischemic insult. Nrf2 was activated by *tert*-butylhydroquinone (tBHQ) intraperitoneally (i.p.) injection (16.7 mg/kg), Nrf2,Trx1 and NLRP3 siRNAs were infused into the left paracele (12 μl per rat), protein and mRNA levels were assessed by Western blot, qRT-PCR. ELISA was used for IL-1β and IL-18 activity measurements. After upregulating Nrf2, the expression of TXNIP in cytoplasm, NLRP3 inflammasome, and downstream factors caspase-1, IL-18, and IL-1β were significantly reduced, and Nrf2 knockdown yielded the opposite results. Trx1 knockdown produced the same effect of Nrf2 inhibition and the protective effect of Nrf2 was mostly abolished. Our results suggested that Nrf2 acted as a protective regulator against NLRP3 inflammasome activation by regulating the Trx1/TXNIP complex, which could possibly represent an innovative insight into the treatment of ischemia and reperfusion injury.

1. Introduction

Stroke is a leading cause of long-term disability and death [1]. Inflammation is one of its critical components in ischemic injury. The nod-like receptor protein 3 (NLRP3) inflammasome is a protein complex located in the cell, and its main function is to activate caspase-1, IL-1β, and IL-18 production [2], then processes apoptosis and inflammation, which causes neuron damage and aggravates brain injury. Recently a few papers reported how the inflammasome was activated, many of them were aimed at the thioredoxin system, Trx1, and TXNIP. Trx1 is a 12-kDa ubiquitous protein [3] that acts as an anti-inflammatory molecule in both extracellular and intracellular environments. TXNIP is a redox-regulated protein, which can bind and directly activate the NLRP3 inflammasome. Zhou et al. [4] found that Trx1 is bound with TXNIP under unstressed conditions, so the inflammasome of NLRP3 remains inactive due to a lack of TXNIP interaction with NLRP3. Under the stress of oxidative conditions such as stroke, the Trx1/TXNIP complex dissociates and then increases the TXNIP/NLRP3 interaction to activate the NLRP3 inflammasome. However, the negative regulator of this activation process has not been fully investigated.

Nrf2 is a widely known transcription factor that plays a critical role in the endogenous antioxidant stress system, which controls the driven genes of antioxidant response element (ARE) [5], and induces Trx1 expression in our previous study [6]. Recent studies have shown that activating the Nrf2 pathway could inhibit NLRP3 inflammasome activation in LPS/GalN-induced liver injury [7]. We hypothesized that Nrf2

Abbreviations: NLRP3, nod-like receptor protein 3; TXNIP, thioredoxin interacting protein; Trx1, thioredoxin1; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element; MCAO, middle cerebral artery occlusion; MCAO/R, middle cerebral artery occlusion/reperfusion; I/R, ischemia reperfusion; tBHQ, *tert*-butylhydroquinone; DMSO, dimethly sulfoxide; TTC, triphenyltetrazolium chloride; qRT-PCR, quantitative reverse transcriptase-PCR

* Corresponding author. Tel.: +86 2368485868.
E-mail address: zjbingsheng@sina.com (J. Zhao).
[1] These authors have contributed equally to this work.

http://dx.doi.org/10.1016/j.bbr.2017.06.027
Received 28 April 2017; Received in revised form 16 June 2017; Accepted 16 June 2017
Available online 26 August 2017
0166-4328/ © 2017 Published by Elsevier B.V.

negatively regulated NLRP3 inflammasome via regulation of the Trx1/TXNIP complex.

In this study, middle cerebral artery occlusion-reperfusion (MCAO/R) model was used, and we aimed to investigate the role of the NLRP3 inflammasome in ischemic/reperfusion injury and whether Nrf2 acted as a negative regulator of NLRP3 inflammasome activation through the Trx1/TXNIP complex.

2. Material and methods

2.1. Animals and agents

All animal procedures were approved by the Institutional Animal Care and Use Committee at Chongqing Medical University. All experimental procedures were performed in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals". Male Sprague-Dawley rats (60–80 d old, 260–300 g) were procured from the Chongqing Medical Animal Experimentation Center. A total of 195 adult male Sprague-Dawley rats were used for the *in vivo* study. All animals were housed under a 12 h light/dark cycle with 23 ± 2 °C temperature and 60–65% humidity, and provided free access to water and food.

2.2. MCAO model

Male Sprague-Dawley rats (260–300 g) had free access to water and food before the operation. Rats were divided randomly into 13 groups: sham, 4 h MCAO, 8 h MCAO, 12 h MCAO, 24 h MCAO, 48 h MCAO, MCAO + control siRNA, MCAO + NLRP3 siRNA, MCAO + Trx1 siRNA, MCAO + Nrf2 siRNA, MCAO + DMSO, MCAO + tBHQ (33.4 mg/kg, intraperitoneal injection), and MCAO + tBHQ + Trx1 siRNA groups. The MCAO model was described in our previous study [8–10]. Rats were deeply anesthetized with chloral hydrate (350 mg/kg, intraperitoneal injection) and then subjected to the operation. A nylon filament (Beijing Cinontech Co. Ltd., Beijing, China) with a rounded tip was placed inside the left middle cerebral artery for 60 min. Then the nylon filament was slowly taken out to restore blood flow to the ischemic artery, which started the reperfusion. Cerebral blood flow was detected by laser Doppler (Periflux System 5000, Perimed AB, Stockholm, Sweden) before ischemia, during MCAO, and during reperfusion. The identical process as was done to the MCAO rats other than the occlusion was performed on the sham-operated rats. Blood reperfusion beneath 70% in rats or rats that expired was removed from the study.

2.3. Preparation and injection of siRNA

The siRNA against NLRP3, Nrf2, and Trx1 was designed by RNA oligochemical synthesis (Genepharma, Shanghai, China). We used 4 NLRP3-rat-siRNA fragments: NLRP3-rat-1980 (sense: 5′-GCUUCAGCCACAUGACUUUTT-3′, antisense: 5′-AAAGUCAUGUGG CUGAAGCTT-3′);

NLRP3-rat-2185 (sense: 5′-CCAGGAGAGAACUUCUUAUTT-3′, antisense: 5′-AUAAGAAGUUCUCUCCUGGTT-3′); NLRP3-rat-2262 (sense: 5′-GGAUCGAAGUGAAAGCCAATT-3′, antisense: 5′-UUGGCUUUCACU UCGAUCCTT-3′); and Negative control (sense: 5′-UUCUCCGAACGU GUCACGUTT-3′, antisense: 5′-ACGUGACACGUUCGGAGAATT-3′). The most significant fragment assayed by Western blot was NLRP3-rat-2185. Nrf2-rat-siRNA (sense: 5′-CCCUGUGUAAAGCUUUCAATT, antisense: 5′-UUGAAAGCUUUACACAGGGTT-3′) fragments and a stable negative control (sense: 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense: 5′-ACGUGACACGUUCGGACAATT-3′) were used. Trx1-rat-siRNA fragments were used (sense: 5′-CCTTCTTTCATTCCCTCTGTGA-3′, antisense: 5′-CCCAACCTTTTGACCCTTTTTA-3′). All siRNAs were dissolved in RNase-free water at a final concentration of 2 μg/uL, oscillated, and centrifuged repeatedly, then infused into the left paracele at the anterior–posterior 0.9 mm, mediolateral 1.9 mm, and dorsoventral 3.5 mm (12 μL/rat), and retained for 10 min. The MCAO model was established 24 h later.

2.4. Treatment of tBHQ

A solution of 100 mg tBHQ *tert*-butylhydroquinone (112941, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 100% dimethly sulfoxide (DMSO) then diluted by phosphate buffered saline (PBS) to a concentration of 5 mg/mL tBHQ, 1% DMSO in PBS. The vehicle solution was the same concentration of DMSO in PBS. The intraperitoneally (i.p.) injection was divided into three times of injections at intervals of 8 h before the MCAO model. Each injection was at a dose of 16.7 mg/kg [11].

2.5. Neurologic deficit evaluation

All sensorimotor scores were evaluated by a blinded investigator after 24 h of reperfusion. The neurological functions were graded according to the Zea–Longa neurological deficit score [12], which is a 5-point spectrum that grades as: 0, no neurological deficit; 1, unable to hold out forepaw completely; 2, unable to move linearly and spiraling to one side; 3, falling to one side; and 4, no spontaneous motor movement.

2.6. Infarct volume measurement

Infarct volume was measured as done in our previous study [6]. After 24 h of reperfusion, all animals were anesthetized and then killed by decapitation and the brain was frozen immediately at −20 °C for 15 min. We then cut the brain into 5 sections and put the brain slices into 2% 2,3,5-triphenyltetrazolium chloride (TTC, T8877 Sigma-Aldrich, St Louis, MO, USA) solution at room temperature for 15 min on each side without light. We then stored them in 4% paraformaldehyde for 24 h. The infarction was quantified by ImageJ software. The infarction (white part) volume was presented as a percentage of the whole volume of the brain area.

2.7. Measurement of IL-18 and IL-1β activity

The level of IL-18 and IL-1β in the cerebral cortex was determined with ELISA kits (Boster Biological Technology, Wuhan, China) as in our previous study [13], according to the manufacturer's instructions and quantified by a microplate reader at 450 nm.

2.8. qRT-PCR

All RNA was extracted from the frozen brain using RNAiso Plus (TaKaRa Biotechnology, Dalian, China) based on the protocol. Reverse transcription was done with a cDNA synthesis kit (TaKaRa Biotechnology). Real-time PCR reactions were performed with TaKaRa SYBR Premix Ex Taq II (Tli RnaseH Plus) (TaKaRa Biotechnology) on a PCR amplifier (CFX-96 Content Real-time System). Primers were listed previously (Sangon Biotech, Shanghai, Co., Ltd.).

2.9. Western immunoblot analysis

Total protein was extracted from the same position of the ischemic side of the rat's cerebral cortex using a commercial kit (Beyotime, China). The nuclear and cytoplasm proteins were extracted using cytoplasmic and nuclear extraction kits (Invent Biotechnologies, USA). Cell lysates were separated by 8%, 10%, and 12% SDS–PAGE and then transferred to PVDF membranes. Then the membranes were obstructed in 5% non-fat milk with TBST buffer for 1 h at room temperature. All membranes were incubated in primary antibody overnight at 4 °C and then in a secondary antibody for 2 h at room temperature. Primary

Download English Version:

https://daneshyari.com/en/article/5735017

Download Persian Version:

https://daneshyari.com/article/5735017

Daneshyari.com