OCCURRENCE OF MITOCHONDRIAL AUTOPHAGY AND NLRP3 INFLAMMATORY BODIES IN CEREBRAL ISCHEMIA-REPERFUSION INJURY AND ITS CORRELATION WITH NEUROINFLAMMATORY RESPONSE

JING YANG[1], YU CHEN[1], YIQIANG PANG[2,*]
[1]Department of Biochemistry and Molecule Biology, Basic Medical Sciences and Forensic Medicine School, Baotou, PR China - [2]Department of Neurosurgery, The fourth Hospital of Baotou, Baotou, PR China

ABSTRACT

Objective: *This article explores the occurrence of mitochondrial autophagy and NOD-like receptor thermal protein domain 3 (NLRP3) inflammatory bodies in cerebral ischemia-reperfusion injury and its correlation with neuroinflammatory responses.*

Methods: *Thirty-five SD rats were collected and adaptively fed for 1 week before the experiment. Fifteen rats were randomly selected as the sham operation group. Meanwhile, the remaining 20 rats were established as a model of cerebral ischemia-reperfusion injury. Model rats were divided into a model group, an Mdivi-1 (mitochondrial autophagy inhibitor) group, a RAPA group (mitochondrial autophagy agonist group) and a siRNA NLRP3 (Intraventricular injection of siRNA in rats, downregulated NLRP3 expression) group, with each group comprising 5 rats. Mitochondrial autophagy and the expression level of cysteine-aspartic protease (Caspase-1), interleukin-1β (IL-1β), NLRP3, COXIV, mitochondrial outer membrane transposase 40 (Tom 40) and manganese superoxide dismutase (MnSOD) were analysed.*

Results: *Mitochondrial autophagy in the model group was significantly stronger than that observed in the sham operation group ($P<0.01$). The number of mitochondria, COXIV, Tom 40 and MnSOD in the model group were significantly lower than was found in the sham group. The expressions of Caspase-1 and IL-1β in the Mdivi-1 group were significantly higher than in the model group, while the expressions of Caspase-1 and IL-1β in the RAPA group were significantly lower than in the model group. The expressions of Caspase-1 and IL-1β in the Mdivi-1 group were significantly higher than seen in the model group, while the expressions of Caspase-1 and IL-1β in the RAPA group were significantly lower than in the model group. The expressions of NLRP3, IL-1β and Caspase-1 in the model group were significantly higher than the sham operation group displayed. Lastly, the expression of Caspase-1 and IL-1β in the siRNA NLRP3 group was significantly lower than that seen in the model group.*

Conclusion: *After cerebral ischemia and reperfusion, mitochondrial autophagy in local brain tissue increases, which can effectively reduce the neuroinflammatory response and relieve neurological deficits. NLRP3 inflammatory bodies can participate in the inflammatory response resulting from cerebral ischemia-reperfusion injury. Downregulating the expression of NLRP3 can inhibit the inflammatory response and promote neural function recovery.*

Keywords: *Cerebral ischemia-reperfusion injury, mitochondrial autophagy, NLRP3 inflammatory bodies, neuroinflammatory response.*

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Introduction

The brain is extremely sensitive to hypoxia. When brain tissue ischemia causes local brain tissue damage and dysfunction, timely restoration of the brain's blood supply can help make the damage reversible, but brain tissue experiencing a state of ischemia for an extended period leads to cerebral infarction[1-2]. Therefore, while it is extremely important to treat patients in a timely and effective manner to restore blood supply to brain tissue, the ischemia-reperfusion injury that occurs during treatment is a problem urgently demanding a solution. Many scholars have conducted in-depth research on the pathophysiological changes and injury mechanisms related to cerebral ischemia-reperfusion inju-

ry. It has been found that the mechanism of cerebral ischemia-reperfusion injury is closely related to the occurrence of mitochondrial autophagy and NOD-like receptor thermo protein domain 3 inflammatory bodies[3-4]. Destruction of mitochondria can lead to the release of large amounts of harmful substances such as reactive oxygen species (ROS), triggering inflammatory reactions and even cell death[5]. Autophagy has the function of degrading excess and old macromolecular substances inside the body's cells and can thereby maintain the homeostasis of the cellular environment[6]. Clinical studies have confirmed that mitochondrial autophagy dysfunction can lead to the occurrence of Parkinson's disease[7].

Moreover, the NLRP3 inflammatory body plays an important role in cerebral ischemia-reperfusion injury, having the ability to recognize many types of pathogens and sense its own danger signals. This capability makes it a critical molecular signalling pathway in the inflammatory cascade[8]. Several studies have confirmed that the expression and structure of NLRP3 inflammatory bodies play a role in NLRP3-mediated inflammatory response and are involved in the development of cerebral ischemia-reperfusion injury[9-10]. Mitochondrial autophagy and the occurrence of NLRP3 inflammatory bodies are closely related to the occurrence of cerebral ischemia-reperfusion injury. Therefore, this study will further investigate the occurrence of mitochondrial autophagy and NLRP3 inflammatory bodies in cerebral ischemia-reperfusion injury and its correlation with neuroinflammatory responses.

Materials and methods

Experimental materials

This study used 35 SD rats (certification number: SCXK (Shanghai) 2019-001, produced by Shanghai Lianmai Biological Engineering Co., Ltd), body weight (270±20) g. All rats were kept in an environment at a temperature of 25oC and 55% humidity. The breeding room was set to offer 12 hours of alternating light/dark conditions, and the rats were fed normally. All rats were adaptively fed for 1 week before the experiment.

Main reagents and instruments

Reagents

NLRP3 was purchased from Shenyang Wanchuang Biotechnology Co., Ltd. Rabbit anti-polyclonal antibody GAPDH was purchased from Guangzhou Weijia Technology Co., Ltd. Luminescent liquid was purchased from Huijingke Biotechnology Co., Ltd. BCA protein quantification kit was purchased from Golden Clone Biotechnology Technology Co., Ltd. RIPA protein lysate was purchased from Harbin Beiguo Haiji Biotechnology Co., Ltd. Goat anti-rabbit HRP secondary antibody was purchased from Boao Parker Biological Company. Polyacrylamide gel kit was purchased from Shanghai Xinle Biological Technology Co., Ltd. Chloraldehyde hydrate was purchased from Nanjing Junke Biological Engineering Co., Ltd.

Instruments

The electrophoresis system was purchased from Shanghai Yihui Biotechnology Co., Ltd. The continuous wavelength microplate reader was purchased from Hangzhou Aosheng Instrument Co., Ltd. The ultra-pure water machine was purchased from Jiangsu Bomeda Life Science Co., Ltd. The ice machine was purchased from Peking University Medical Industry Technology Co., Ltd. The anaesthesia tank (sevoflurane) was purchased from Shenzhen Ruiwode Life Technology Co., Ltd. The thermostatic oscillator was purchased from Wuxi Microchromatography Biotechnology Co., Ltd. The thermostatic metal bath was purchased from Guangzhou Dahui Biotechnology Co., Ltd. The dissecting microscope was purchased from Dongguan Spectrum Standard Experimental Equipment Technology Co., Ltd. The experimental animal thermostatic operating table was purchased from Hong Kong Biotech Co., Ltd. The rat brain section fixation groove was purchased from Hunan Yuanxiang Biological Technology Co., Ltd. The inhalation anaesthesia machine was purchased from Henan Zhike Hongrun Environmental Protection Technology Co., Ltd.

Methods

• Fifteen rats were randomly selected as the sham operation group, and the remaining 20 rats were used to establish a cerebral ischemia-reperfusion injury model as the model.

A middle cerebral artery occlusion operation was performed on both groups of rats, and no thread plug was placed in rats from the sham operation group. After the operation, the two groups of rats were raised in different cages. The model rats were divided into 4 groups of 5 rats each: a model group, an Mdivi-1 group, a RAPA group and a siRNA NLRP3 group. All rats were sacrificed and their brain tissues were obtained.

• Electron microscopy was used to detect mitochondrial autophagy in rats from the sham operation group and the model group.

• Western blot was used to detect the expression levels of rat cysteine-aspartic protease (Caspase-1), interleukin-1β (IL-1β), NLRP3, COXIV and mitochondrial outer membrane transposase 40 (Tom 40) along with manganese superoxide dismutase (Mn-SOD).

Statistical methods

The autophagy and neuritis of rats in each group were expressed by $\bar{x}\pm s$. Differences between groups were tested by t-test. Two-way ANVOA software was used to analyse the data. $P<0.05$ was considered a significant difference.

Results

Comparison of mitochondrial autophagy in each group of rats

Mitochondrial autophagy in the model group was significantly stronger than in the sham operation group ($P<0.01$) as seen in Figure 1.

The number of mitochondria along with COX-IV, Tom 40 and MnSOD expression in the model group were significantly lower than was observed in the sham operation group. See Table 1.

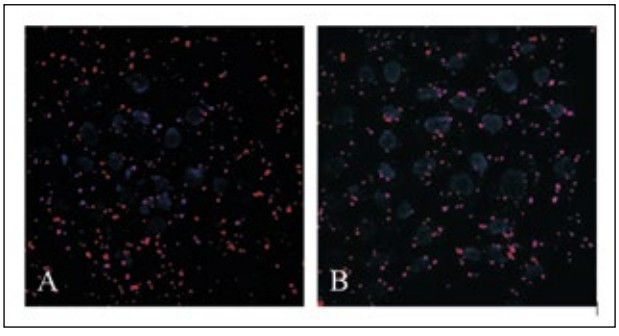

Figure 1: Comparison of mitochondrial autophagy in each group of rats.
Note: A: sham operation group; B: model group.

Group	Number of mitochondria (case)	COXIV expression	TOM40 expression	MnSOD expression
Mock surgical group	25.00±2.00	0.89±0.31	0.92±0.08	0.91±0.07
Model group	14.00±1.00	0.25±0.16	0.61±0.12	0.42±0.11
t	19.053	7.105	8.325	14.555
P	<0.001	<0.001	<0.001	<0.001

Table 1: Comparison of mitochondrial autophagy in each group of rats ($\bar{x}\pm s$).

The role of mitochondrial autophagy and neuroinflammatory response to cerebral ischemia-reperfusion injury

The expressions of Caspase-1 and IL-1β in the Mdivi-1 group were significantly higher than observed in the model group, while the expressions of Caspase-1 and IL-1β in the RAPA group were significantly lower than in the model group as seen in Figure 2.

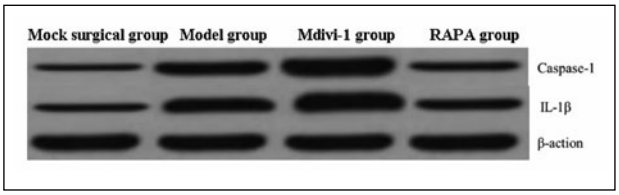

Figure 2: Effect of mitochondrial autophagy and neuroinflammatory response to cerebral ischemia-reperfusion injury.

Comparison of NLRP3-mediated neuroinflammatory responses in rats from each group

The expressions of NLRP3, IL-1β and Caspase-1 in the model group were significantly higher than shown in the sham operation group as can be seen in Table 2.

Group	NLRP3 expression	IL-1β expression	Caspase-1 expression
Mock surgical group	25.65±3.01	1.01±0.01	1.01±0.03
Model group	45.89±4.56	2.03±0.35	3.24±1.13
t	14.347	17.538	4.411
P	<0.001	<0.001	0.002

Table 2: Comparison of NLRP3-mediated neuroinflammatory responses in rats from each group ($\bar{x}\pm s$).

Effect of downregulation of NLRP3 expression on cerebral ischemia-reperfusion injury

The expression of Caspase-1 and IL-1β in the siRNA NLRP3 group was significantly lower than that observed in the model group as seen in Table 3.

Group	IL-1β expression	Caspase-1expression
Mock surgical group	1.01±0.01	1.01±0.03
Model group	2.03±0.35	3.24±1.13
siRNA NLRP3	0.63±0.08	0.75±0.01
t	27.868	27.175
P	<0.001	<0.001

Table 3: Effect of downregulation of NLRP3 expression on cerebral ischemia-reperfusion injury ($\bar{x}\pm s$).

Discussion

The increasing incidence of cerebral ischemia-reperfusion injury offers a significant threat to human life and health. Moreover, its pathophysiological process is extremely complicated, a key factor in the current lack of effective treatment measures[1]. Neuroinflammation, an essential process of cerebral ischemia-reperfusion injury, plays an important role in secondary neuron death[11]. Long-lasting inflammation after cerebral ischemia-reperfusion injury is a significant factor in acute nerve injury after cerebral ischemia-reperfusion injury[12]. Therefore, it is important to understand the specific mechanism of cerebral ischemia-reperfusion injury in developing new treatments.

Mitochondrial autophagy is an important means for mitochondrial renewal and metabolism. It can regulate the number and quality of mitochondria and induce mitochondria to maintain relative balance[13-14]. Several studies have confirmed that mitochondrial autophagy is significantly enhanced after cerebral ischemia-reperfusion injury[15]. Inflammatory bodies can integrate relevant inflammatory factor precursors and then trigger an inflammatory response, which is the body's innate immune defence function. In addition, under pathological conditions, inflammatory bodies can promote the inflammatory death function of cells that Caspase-1 is dependent on, eventually inducing programmed cell death[16-17]. An improperly regulated NLRP3 inflammatory body will easily cause an excessive inflammatory response, inducing IL-1β, IL-18 and other inflammatory factors to secrete, activating downstream signalling pathways and eventually leading to an inflammatory cascade[18-19]. NLRP3 inflammatory bodies are regulated by many substances and signalling pathways. Clinical studies have shown that NLRP3 inflammatory bodies participate in the onset and progression of diseases such as Parkinson's by inducing a neuroinflammatory cascade[20].

This study established a rat ischemia-reperfusion model and observed the occurrence of mitochondrial autophagy and inflammatory bodies. The results showed that mitochondrial autophagy in the model group was significantly stronger than it was for the sham operation group ($P<0.01$).

The number of mitochondria as well as COX-IV, Tom 40 and MnSOD expression in the model group were significantly lower than in the sham operation group. This indicates that mitochondrial autophagy is significantly enhanced after cerebral ischemia-reperfusion injury, which can promote the elimination of damaged mitochondria. The expressions of Caspase-1 and IL-1β in the Mdivi-1 group were significantly higher than in the model group, while the expressions of Caspase-1 and IL-1β in the RAPA group were significantly lower than those seen in the model group. The expressions of Caspase-1 and IL-1β in the Mdivi-1 group were significantly higher than those the model group showed, while the expressions of Caspase-1 and IL-1β in the RAPA group were significantly lower than in the model group. These results indicate that obstructing mitochondrial autophagy after cerebral ischemia-reperfusion injury will lead to further aggravation of the neuroinflammatory response.

To a certain extent, strengthening mitochondrial autophagy is beneficial to inhibiting the neuroinflammatory response. The expressions of NLRP3, IL-1β and Caspase-1 in the model group were significantly higher than the sham operation group, suggesting that the NLRP3 inflammatory bodies are activated after cerebral ischemia-reperfusion injury, which promotes the release of related inflammatory mediators and eventually induces the occurrence of inflammatory reactions. The expression of Caspase-1 and IL-1β in the siRNA NLRP3 group was significantly lower than that observed in the model group, which indicates that downregulating the expression of NLRP3 after cerebral ischemia-reperfusion injury can effectively control the expression of inflammatory mediators, thereby inhibiting excessive activation of neuroinflammation.

In summary, after cerebral ischemia and reperfusion, mitochondrial autophagy in local brain tissue was increased, effectively reducing the neuroinflammatory response and relieving neurological deficits. While NLRP3 inflammatory bodies may participate in the inflammatory response caused by cerebral ischemia-reperfusion injury, downregulation of NLRP3's expression can inhibit the inflammatory response and promote neural function recovery.

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Corresponding Author:
Lin Chen
Email: eauy29@163.com
(China)