



Effect of baicalin on the autophagy and Beclin-1 expression in rats with cerebral ischemia

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ABSTRACT

Objective: To explore the effect of baicalin on the autophagy and Beclin-1 expression in rats with cerebral ischemia, and the role of autophagy in the cerebral ischemia injury. **Methods:** The healthy male SD rats were randomized into the sham operation group, the ischemia model group, baicalin treatment group (100 mg/kg), and 3MA group (15 mg/kg), with 10 rats in each group. Transient focal cerebral ischemia injury model in rats was induced by occlusion of middle cerebral artery (MCA) for 180 min. The rats were given the corresponding drugs through the tail veins 30 min before molding. Half of the specimens were used for TTC staining to analyze the cerebral infarction volume. The others were used to determine the expression of Beclin-1 in the brain tissues by Western-blot. **Results:** When compared with the ischemia model group, the cerebral infarction volume in 3MA group was significantly increased, while that in baicalin treatment group was significantly reduced, and the comparison among the groups was statistically significant. When compared with the ischemia model group, Beclin-1 expression level in baicalin treatment group was significantly elevated, while Beclin-1 expression level in 3MA group was significantly higher than that in the sham-operation group but lower than that in the ischemia model group. **Conclusions:** The autophagy level of brain tissues in normal rats is low. The cerebral ischemia can activate autophagy. The activated autophagy is probably involved in the neuroprotection of cerebral ischemia injury. Application of 3MA to inhibit the occurrence of autophagy can aggravate the cerebral injury. Baicalin can significantly improve the cerebral ischemia injury and promote the occurrence of autophagy, whose mechanism is probably associated with the up-regulation of Beclin-1 expression to promote the activation of type III PI3K signal transduction pathway.

1. Introduction

The ischemic cerebrovascular disease is a kind of disease which can endanger the human health. The cerebral ischemia is a kind of pathological and physiological process of irreversible injury of neurons caused by inadequate brain tissue blood flow, which can affect the neurological function[1]. Some researches demonstrate that[2] autophagy is also involved in the occurrence and development of cerebral injury. Baicalin, a kind of flavonoids, extracted from the

traditional Chinese medicine *Scutellaria Baicalensis* Georgi with the highest content, has the anti-bacterial, anti-inflammation, and anti-oxidation effects[3]. The study is aimed to explore the effect of baicalin on the autophagy and Beclin-1 expression in rats with cerebral ischemia in order to provide a theoretical evidence for a wide application in the clinic.

2. Materials and methods

2.1. Materials

2.1.1. Experiment animals

A total of 40 male SD rats weighing 250-300 g were obtained from the Experimental Animal Center of Medical College of Shantou University (Certification No. SCXK(Yue)2012_0012). In order to

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control the blood sugar level, the rats were fasted for 12-24 h before experiment, but water was not forbidden.

2.1.2. Experiment reagents

Baicalin, with a purity degree >98%, was purchased from Chinese Pharmaceutical Biological Products Assay Institute (Batch No. 20140309). The normal saline was dissolved for reserve. 3-Methyladenine (3MA) was purchased from Cayman. 2,3,5-TTC was purchased from Wuhan Boster Bioengineering Co. Ltd., and was prepared in 2% solution when using. Anti-BECN1(Beclin-1) was purchased from Abcam. GAPDH was purchased from Santa Cruz.

2.2. Methods

2.2.1. Experiment grouping and administration

The healthy male SD rats were randomized into the sham operation group, the ischemia model group, baicalin treatment group (100 mg/kg), and 3MA group (15 mg/kg), with 10 rats in each group. The rats were given the corresponding drugs through the tail veins 30 min before molding. The rats in the sham operation group and the ischemia model group were given the equal normal saline. Half of the specimens were used for TTC staining to analyze the cerebral infarction volume. The others were used to determine the expression of Beclin-1 in the brain tissues by Western-blot.

2.2.2. Model preparation

Intraperitoneal injection of 2% pentobarbital sodium (4 mg/100 g) was used for the anesthesia. A supine position was taken. The model was made according to the improved molding method reported by Xiao et al[4]. A cervical median incision was made to separate Common carotid artery (CCA), External carotid artery (ECA), and Internal carotid artery (ICA). ECA and its branches (occipital artery, superior thyroid artery, ascending pharyngeal artery, lingual artery, maxillary artery, and pterygopalatine artery) were ligated and blocked. No. 4-0 nylon thread coated with silica gel with a diameter of 0.25 mm was taken as the embolism thread to insert from the stub end of ECA to the bifurcation of CCA, and then to ICA in a depth of about (18.0±0.5) mm. If the resistance was increased, the embolism thread was inserted into the initial part of Middle cerebral artery (MCA), which could block the blood supply of MCA to cause local cerebral ischemia. During the process of artery separation, the vagus nerves along ECA and CCA should be protected to avoid stimulating the trachea, and reduce the traction as much as possible. And then the wound was sutured. After MCA blocked for 180 min, if Horner's syndrome on the right side was positive, the left forelimb was bended when lifting the tail, the body drew circles to the left side when automatically moving, so that the molding was successful. Longa method[5] was used to evaluate the ischemia behavior. In the sham operation group, CCA, ECA, and ICA were separated, the blood supply was not blocked, and then the subcutaneous tissues and skin were sutured.

2.2.3. TTC staining

After ischemia for 180 min, the head was beheaded for the brain tissues. After frozen at -80 °C, the brain tissues were sliced into

coronal pieces with a thickness of 2 mm, placed in 2% TTC solution, and incubated at 37 °C away from the light for 30 min. In order to keep the staining even, the slices were overturned every 10 min. After staining, the slices were placed in 0.01 mmol/L PBS solution, and the images were collected. The normal tissues were stained in red, while the infarction tissues were not stained. Luxex-F image analyzer was used to analyze the infarct volume. The constant ipsilateral frontal-partial cortex infarction could be seen in the infarction area.

2.2.4. Western-blot method

After ischemia for 180 min, the head was beheaded for the brain tissues which were washed with precooled normal saline. The liquid nitrogen was ground. The protein lysis solution was used to split the brain tissues for protein quantification. A routine SDS-PAGE electrophoresis was performed. After the membrane was transferred for 1 hour and 30 min with a transverse flow of 100 mA, it was sealed for 2 h. Beclin-1 antibody was added and incubated at 4 °C overnight. The second antibody (goat anti-rabbit, 1:1 000) was incubated at room temperature for 1 h. ECL luminous fluid was used for shining. The film was exposed for developing. β -actin was taken as internal reference. Gel image system was used to analyze the expression quantity.

2.3. Statistical analysis

SPSS 19.0 software was used for the statistical analysis. The measurement data were expressed as mean \pm SD. T test was used for the comparison among the groups. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. Comparison of the cerebral infarction volume after TTC staining among the groups

When compared with the ischemia model group, the cerebral infarction volume in 3MA group was significantly increased, while that in baicalin treatment group was significantly reduced, and the comparison among the groups was statistically significant ($P < 0.05$) (Table 1).

Table 1

Comparison of the cerebral infarction volume after TTC staining among the groups (mean \pm SD).

Groups	n	Dosage (mg/kg)	Cerebral infarction volume (mm ³)
Sham-operation	5	-	-
Ischemia model	5	-	198.69±33.15
Baicalin treatment	5	100	150.92±47.22*
3MA	5	15	236.84±40.52*

* $P < 0.05$, when compared with the ischemia model group.

3.2. Comparison of Beclin-1 expression level in the brain tissues among the groups

Beclin-1 expression level in the normal brain tissues was significantly lower than that in the other 3 groups ($P < 0.05$). Beclin-1

expression level in the ischemia model group was significantly higher than that in the sham-operation group ($P<0.05$). Beclin-1 expression level in the baicalin treatment group was significantly higher than that in the ischemia model group ($P<0.05$). Beclin-1 expression level in 3MA group was significantly lower than that in ischemia model group and baicalin treatment group ($P<0.05$) (Table 2).

Table 2

Comparison of Beclin-1 expression level in the brain tissues among the groups (mean±SD).

Groups	Dosage (mg/kg)	Expression level (Resolution)
Sham-operation	-	0.613±0.193
Ischemia model	-	2.942±0.461**
Baicalin treatment	100	3.274±0.501*
3MA	15	1.309±0.227*

** $P<0.05$, when compared with the sham-operation group; * $P<0.05$, when compared with the ischemia model group.

4. Discussion

During the process of cerebral ischemia injury, the blood supply is inadequate and the microcirculation is blocked, which can induce a series of pathological and physiological alterations and cell metabolic disorders, finally resulting in neurons damaged, which can severely affect the brain function[6].

Among the eukaryotic cells, autophagy is a kind of adaptive response in lack of nutrition, and is involved in various pathological and physiological process of mammals[7], which plays an important role in maintaining the homeostasis. Some researches demonstrate that[8] when the cells are stimulated by hypoxia, high temperature, hungry, and other external factors, or excessive damaged cytoplasm ingredient accumulation, and other internal factors, the autophagy can be induced. The autophagy can maintain in a basic level under a physiological condition. Some other researches demonstrate that[9] in a condition of ischemia and hypoxia, a large amount of autophagy can be formed in the brain tissues of adult rats, showing that the brain tissue ischemia and hypoxia is a forceful stimulation factor for developing autophagy. Beclin-1 is a homologous gene with Atg6 in the mammal cells, is combined with type III PI3K to be involved in the formation of autophagosome, and regulate the positioning of other Atg proteins in the autophagy precursor structures, which plays an important role in the genesis and development of autophagy[10]. Beclin-1 is a key protein to regulate the activity of autophagy, and is a specific gene to be involved in the autophagy of mammals[11]. Some scholars argue that[12] the newborn 7dSD rats ischemia and hypoxia experiment shows that the autophagy can be activated a short time after ischemia, application of 3MA can increase the amount of necrotic cells due to ischemia and hypoxia, while application of autophagy activator rapamycin can reduce the necrosis of cells, which can alleviate the cerebral injury to a certain extent. The results in the study showed that application of 3MA can reduce the expression level of Beclin-1 in rats with cerebral ischemia injury, probably through inhibiting the activation of type III PI3K to reduce the autophagy level, while the cerebral infarction

scope was increased, showing that inhibition of autophagy can aggravate the cerebral ischemia injury to a certain extent, which is probably associated with the protection effect of autophagy on the neurons during the ischemia period. Baicalin, a kind of flavonoids, extracted from the dry roots of *Scutellaria Baicalensis* Georgi, has heat clearing and toxicity removing, sedation and anti-inflammation, and anti-oxidation effects[1]. The results in the study showed that baicalin can significantly shrink the cerebral ischemia infarction scope, improve the neurological dysfunction caused by local cerebral ischemia injury, and increase the expression level of Beclin-1 to promote the occurrence of autophagy to a certain extent when compared with the pure cerebral ischemia.

In conclusion, autophagy can protect the damaged neurons to a certain extent during the ischemia period. Baicalin can significantly improve the cerebral ischemia injury and promote the occurrence of autophagy, whose mechanism is probably associated with the up-regulation of Beclin-1 expression to promote the activation of type III PI3K signal transduction pathway; therefore, a further study on its mechanism is required.

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