XingNaoJing injection ameliorates cerebral ischaemia/reperfusion injury via SIRT1-mediated inflammatory response inhibition

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ABSTRACT

Context: XingNaoJing injection (XNJ), extracted from a traditional compound Chinese medicine Angong niuhuang pill, is well known for treating stroke in the clinic, but the specific effects and mechanisms remain unclear.

Objective: We investigated the mechanistic basis for the protective effect of XNJ on cerebral ischaemia/reperfusion (I/R) injury.

Materials and methods: Five groups of 10 SD rats underwent 2 h of middle cerebral artery occlusion (MCAO) followed by 24 h reperfusion. XNJ at 10 and 15 mL/kg was intraperitoneally administered 24 h before ischaemia and at the onset of reperfusion respectively. The silent information regulator 1 (SIRT1) inhibitor EX527 was intracerebroventricularly injected 0.5 h before reperfusion. Cerebral infarction size, neurological scores, morphological changes, and expression levels of inflammatory mediators and SIRT1 were measured. Furthermore, human brain microvascular endothelial cells (HBMECs) were subjected to 3 h oxygen and glucose deprivation (OGD) followed by 24 h reoxygenation to mimic cerebral I/R in vitro. EX527 pre-treatment occurred 1 h before OGD. SIRT1 and inflammatory mediator levels were analyzed.

Results: Both XNJ doses significantly decreased cerebral infarct area (40.11% vs. 19.66% and 9.87%) and improved neurological scores and morphological changes. Inflammatory mediator levels were remarkably decreased in both model systems after XNJ treatment. XNJ also enhanced SIRT1 expression. Notably, the SIRT1 inhibitor EX527 attenuated the XNJ-mediated decrease in inflammation in vivo and in vitro.

Conclusions: XNJ improved cerebral I/R injury through inhibiting the inflammatory response via the SIRT1 pathway, which may be a useful target in treating cerebral I/R injury.

Introduction

Ischaemic stroke remains a major cause of acquired adult disability and mortality worldwide. Reperfusion is the major approach to treat ischaemic stroke; however, it can cause serious secondary brain tissue injury, namely cerebral ischaemia/reperfusion (I/R) injury. A wave of pathologic events occurs during cerebral I/R injury, including energy metabolism impairment, oxidative stress, glutamate/neurotoxin release, calcium and sodium overload, apoptosis, inflammation, and autophagy (Fann et al. 2013; Radak et al. 2017; Wu J et al. 2017; Huang et al. 2018). Among these mechanisms, inflammation is considered a key element in the complicated pathogenesis of cerebral I/R injury.

Inflammation is a dynamic process that mainly consists of activation of microglia and transcriptional factor nuclear factor kappa B (NF-kB)/p65, which initiates the production of adhesion molecules such as intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), as well the release of pro-inflammatory cytokines tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and IL-6 by activating endogenous macrophages (Kettenmann and Verkhratsky 2008; Tu et al. 2010; Duris et al. 2018). In its inactive state, the most common form of NF-kB is the p50-p65 heterodimer that forms a complex with its repressor I-kappa-B-alpha (IκBα) in the cytoplasm (Chen et al. 1999). Following specific stimuli, IκBα is degraded, and the heterodimer translocates to the nucleus, affecting transcription. Adhesive molecules attract inflammatory mediators to the affected area, leading to leukocyte infiltration and subsequent promotion of the inflammatory response. Many studies have demonstrated that inflammation plays an important role in the development of cerebral ischaemia (Su et al. 2017; Gray et al. 2018; Xu et al. 2018). Hence, appropriate inhibition of inflammation is a beneficial strategy to protect against cerebral I/R injury. Silent information regulator 1 (SIRT1) is also involved in protecting against brain ischaemia (Meng et al. 2017). It is a member of the class III histone/protein deacetylases and has anti-inflammatory effects in many diseases (He et al. 2017; Koo and Cho 2017; Escribano-Lopez et al. 2018; Zhu et al. 2019).

XNJ injection is extracted from the Angong niuhuang pill, a traditional compound Chinese medicine widely used to treat stroke in China with the approval of the Chinese National Drug Administration (Guo et al. 2014). XNJ is a herbal preparation of Moschus (Moschus berezovskii Florov [Cervidae]; 7.5 g), Radix curcumae (Curcuma wenyujin Y.H.Chen and C.Ling [Zingiberaceae]; 30 g), Borneolum (Blumea balsamifera DC [Compositae]; 1 g) and Fructus gardenia (Gardenia jasminoides J.Ellis [Rubiaceae]; 30 g). The quality control standard of XNJ is very strict and was described in our previous study (Zhang et al. 2020, 2019; Duris et al. 2018). It is a traditional compound Chinese medicine widely used to treat stroke in China with the approval of the Chinese National Drug Administration (Guo et al. 2014). XNJ is a herbal preparation of Moschus (Moschus berezovskii Florov [Cervidae]; 7.5 g), Radix curcumae (Curcuma wenyujin Y.H.Chen and C.Ling [Zingiberaceae]; 30 g), Borneolum (Blumea balsamifera DC [Compositae]; 1 g) and Fructus gardenia (Gardenia jasminoides J.Ellis [Rubiaceae]; 30 g). The quality control standard of XNJ is very strict and was described in our previous study (Zhang et al. 2020, 2019; Duris et al. 2018).
Firstly, curzerenone was used to standardize XNJ, and the fingerprint showed that the high-performance liquid chromatography retention time for curzerenone in XNJ was consistent with that of standard curzerenone. Muscone and borneol were also used as quality control substances for gas chromatograph analysis; muscone should be no less than 0.1 mg and borneol should be between 0.8 and 1.2 mg in every millilitre of XNJ. Quality control results confirm the reliability of clinical XNJ preparations. Both clinical trials and basic experiments have shown that XNJ can improve brain injury, promote consciousness recovery, and provide neuroprotective effects in stroke (Xu et al. 2014; Wu L et al. 2016; Ma et al. 2017; Zhang et al. 2018). However, the specific effects and mechanisms of XNJ on the cerebral I/R remains unclear, which limits further clinical application. Here, we used both in vivo and in vitro models to investigate whether SIRT1-dependent inflammation repression is involved in the effects of XNJ on cerebral I/R injury.

Materials and methods

Reagents

XNJ (Chinese Food and Drug Administration number z41020664) was purchased from Henan Tiandi Pharmaceutical Co., Ltd. (Henan, China). 2,3,5-Triphenyltetrazolium chloride (TTC), haematoxylin and eosin (H&E) staining kits and SIRT1 activity assay kit were provided by Sigma (St. Louis, MO, USA). The antibody against SIRT1 was purchased from Cell Signalling Technology (CST, Danvers, MA, USA). Antibodies against NF-κB/p65, ICAM-1, VCAM-1, and β-actin and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Beyotime Institute of Biotechnology (Beijing, China). Antibodies against TNF-α, IL-6, and IL-1β were obtained from Santa Cruz Biotechnologies (Dallas, TX, USA). EX527 was purchased from Selleckchem (Houston, TX, USA). All other reagents were from common commercial sources.

Animal and study design

Adult male Sprague-Dawley rats (250–280 g) purchased from Jilin University Animal Centre were treated based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 2011). All experimental procedures applied in this research were approved by the Animal Ethics Committee of Jilin University. Every effort was made to minimize the pain of animals. Rats (N = 50) were randomly divided to 5 groups of 10 rats each: Sham, I/R, I/R + XNJ (10 or 15 mL/kg), and I/R + XNJ (15 mL/kg) + EX527. The doses were selected in accordance with the clinical use of XNJ. Human doses of XNJ were converted to rat doses according to a conversion coefficient (0.018) based on the body surface area. A focal cerebral I/R model was accomplished by middle cerebral artery occlusion (MCAO) performed under a heating lamp at 37°C as previously described (Longa et al. 1989). Briefly, a 2 cm midline incision was operated on the neck to expose the right common, external, and internal carotid arteries. Next, we ligated the external carotid artery branches at 2.0 mm from the bifurcation of the common carotid artery, and a standard 4-0 nylon filament with a heat-blunted tip was carefully inserted 18–20 mm from the incision to block the right middle cerebral artery for 2 h. Afterward, the filament was withdrawn gently to restore blood flow and allow 24 h reperfusion. The sham group underwent the same surgical operations except occlusion/reperfusion. Two doses of XNJ were injected intraperitoneally 24 h before the ischaemia procedure and at the onset of reperfusion. EX527 (10 mM) dissolved in dimethyl sulfoxide (DMSO) was injected into the right lateral ventricle 0.5 h before reperfusion. Sham and I/R rats received an equivalent volume of saline and DMSO.

Estimation of the neurological score

Neurological deficit scores were estimated blindly using a 5-point scale as previously described (Longa et al. 1989; Frank-Cannon et al. 2009). The scoring criteria were as follows: without obvious neurological deficits, grade 0; unable to fully extend contralateral forepaw, grade 1; circling continuously to the contralateral side, grade 2; falling to the left, grade 3; falling to move spontaneously and loss of consciousness, grade 4; and death associated with cerebral ischaemia, grade 5. Injury severity is inversely proportional to the score.

Assessment of infarct volume

After collecting the brain tissues, a brain slicer matrix was used to cut tissues into five 2 μm thick coronal sections. Then the sections were incubated in 2% TTC (Sigma-Aldrich) at 37°C for 0.5 h and photographed with a digital camera. The ratio of infarct area was quantified by ImageJ software (NIH, Bethesda, MD, USA).

Histopathology and immunohistochemistry

After 24 h of reperfusion, the rats were euthanized, and brain tissues were fixed in 4% buffered formaldehyde overnight. Then specimens were embedded in paraffin blocks and prepared for sectioning into 5 μm thick slices. For histopathology, the obtained tissue sections were deparaffinized and stained with H&E according to standard protocols. For immunohistochemistry, sections were incubated with anti-SIRT1 primary antibody and anti-rabbit IgG secondary antibody in combination with 3,3′-diaminobenzidine chromogen (DAB) followed by haematoxylin counterstain. All preparations were analyzed under a light microscope to detect morphologic changes (Olympus, Tokyo, Japan). Ten areas of each slide were observed at 100× or 400× magnification.

SIRT1 activity assays

The SIRT1 activity was quantified using a SIRT1 fluorometric assay kit Fluorometric Assay Kit according to the manufacturer’s instruction.

Cell culture and OGD

HBMECs (purchased from Chinese Academy of Medical Sciences) were cultured in DMEM supplemented with 10% foetal bovine serum (FBS, Gibco, Gaithersburg, MD USA). Cells were allocated randomly to 4 groups: control, OGD, OGD + XNJ (2.5 μL/mL), OGD + XNJ (2.5 μL/mL) + EX527 (2 μM). OGD was performed by culturing the cells with glucose and serum-free basic salt solution (BSS, 5 mmol/L KCl, 120 mmol/L NaCl, 1.2 mmol/L CaCl2, 1.1 mmol/L KH₂PO₄, 20 mmol/L Na₂CO₃, and 1.2 mmol/L MgSO₄). Next the plate was placed in an anaerobic chamber at 37°C with a controlled atmosphere of 5% CO₂, 85% N₂, and 10% H₂ for 3 h. Finally, BSS was replaced with...
standard medium, and the cells returned to the normal incubator with 5% CO₂ for 24 h to recover.

**Western blot analysis**

Radioimmunoprecipitation assay lysis buffer with 10% protease inhibitor phenylmethylsulphonyl fluoride was used to extract total protein from brain samples (right hemisphere). Protein contents were measured using a bicinchoninic acid protein assay kit (Beyotime Biotechnology, Beijing, China). Protein extracts (20 μg/lane) were separated on by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes. At the end of transfer, the membranes were blocked with 5% non-fat milk in Tris-buffered saline and Tween 20 (pH 7.6) for 1 h at room temperature. Then membranes were incubated with anti-SIRT1, NF-κB/p65, ICAM-1, VCAM-1, TNF-α, IL-1β, IL-6, and β-actin antibodies overnight at 4°C and appropriate secondary antibody for 1 h at room temperature. Next, enhanced chemiluminescence reagent was added to membranes to visualise protein bands that were quantified the Quantity One software package (West Berkeley, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase and β-actin were used for standardization.

**Immunofluorescent assay**

After experimental procedures, cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. Then cells were blocked with 5% bovine serum albumin, and the primary antibody was added to cells at 4°C overnight. Fluorescein isothiocyanate-labelled secondary antibody incubation occurred in the dark for 1 h followed by DAPI staining for 5 min. A fluorescence microscope (Olympus) was used to assess positive staining.

**Statistical analysis**

Data are expressed as mean ± standard error of the mean (SEM). All statistical analyses were performed using GraphPad Prism software (version 6.0; GraphPad Software Inc., San Diego, CA, USA). Statistical differences among multiple groups were determined by one-way analysis of variance followed by Dunnett’s test or Student’s t-test. Statistical significance was established when \( p < 0.05 \).

**Results**

**XNJ decreased brain infarct volume and improved neurological function in cerebral I/R injury rats**

To investigate the effect of XNJ on cerebral I/R outcomes, we detected neurological scores and cerebral infarct volume 24 h after reperfusion. The Longa scores showed that the I/R group exhibited obvious neurological dysfunction compared with the Sham group, indicating that the rat cerebral I/R model was successfully established. XNJ treatment obviously improved neurological deficits (Figure 1(A)). Moreover, TTC staining confirmed that rats in the I/R + XNJ group had smaller infarcts in the cortex and striatum compared to the I/R group: infarction areas in I/R + XNJ groups were 19.66% and 9.87%, and significantly lower than I/R group (40.11%) (Figure 1(B,C)). The observed reduction in neurological deficit scores and infarct volume demonstrated that cerebral I/R injury could be mitigated by XNJ treatment.

**XNJ inhibited morphologic injury in cerebral I/R injury rats**

To further confirm the effects of XNJ treatment on I/R brain injury, we analyzed morphology changes by H&E staining the cerebral cortex, white matter, and hippocampus and found similar results as observed with TTC. In the I/R group, neuronal cells of the cortex developed pyknotic nuclei and vacuoles around the nuclei. XNJ attenuated these changes (Figure 2(A)). Consistently, white matter leukoaraiosis was observed in the I/R group and was alleviated by XNJ treatment (Figure 2(B)). Figure 2(C) shows cell shrinkage with condensed nuclei and neuron loss in the hippocampus after I/R, but these changes were prevented by XNJ. No morphological changes were observed in the cortex, white matter, or hippocampus in the sham group.

**XNJ prevented neuroinflammation during cerebral I/R injury in rats**

Inflammation plays a vital role in cerebral I/R injury. To determine the effect of XNJ on inflammation, protein levels of activated NF-κB/p65, 1κBα, adhesion molecules, and relative inflammatory factors were measured by western blot analysis. As shown in Figure 3, I/R significantly elevated levels of phosphorylated NF-κB/p65, VCAM-1, ICAM-1, TNF-α, IL-1β, and IL-6 and decreased the level of 1κBα; these increases were significantly

![Figure 1](image-url). Effects of XNJ on brain infarct area and neurological scores. (A) XNJ obviously improved neurological scores in comparation with the I/R group. Data are represented as mean ± S.E.M. (n = 10). (B) Representative TTC staining section. (C) Quantitative analysis of infarct regions. Brain infarct area was distinctly decreased after XNJ treatment in comparation with the I/R group (n = 3). (**p < 0.001 vs. Sham; #p < 0.05 vs. I/R; ##p < 0.01 vs. I/R; ###p < 0.001 vs. I/R).
attenuated by XNJ treatment. The results suggested that XNJ may relieve cerebral I/R damage by inhibiting the inflammatory response.

**XNJ increased SIRT1 expression and activity during cerebral I/R injury in rats**

To explore the effect of XNJ on SIRT1 expression 24 h after cerebral I/R, we performed immunohistochemistry to measure the protein levels of SIRT1. Expression of SIRT1 decreased under after I/R, but higher levels were observed in the XNJ group compared to the I/R group (Figure 4(A,B)). Western blotting results were consistent with those of immunohistochemistry (Figure 4(C)). The SIRT1 activity was determined using a SIRT1 fluorometric assay kit. XNJ treatment significantly increased SIRT1 activity compared to the I/R group (Figure 4(D)). These findings demonstrate that SIRT1 expression was increased by XNJ in the rat cerebral I/R injury model.

**XNJ-regulated inhibition of the inflammatory response in cerebral I/R injury rats was mediated by SIRT1**

To investigate whether SIRT1 was involved in regulating inflammation in XNJ-treated cerebral I/R rats, we used the specific SIRT1 inhibitor EX527 to block its activity. As expected, compared with the I/R+XNJ groups, EX527 significantly reversed
Figure 3. XNJ treatment blocked the expression of inflammatory mediators in cerebral I/R injury in rats. (A) Representative western blots showing protein levels of NF-κB/p65, IκB-α, VCAM-1, ICAM-1, TNF-α, IL-1β and IL-6. Protein levels were standardized to β-actin. Quantification of protein levels were shown in (B, C, D, E, F, G). Data are represented as mean ± S.E.M. (*p < 0.05 vs. Sham; **p < 0.01 vs. Sham; ***p < 0.001 vs. Sham; #p < 0.05 vs. I/R; ##p < 0.01 vs. I/R; ###p < 0.001 vs. I/R). n = 4 in each group.

Figure 4. The effects of XNJ on SIRT1 expression and activity in cerebral I/R injury in rats. (A) Representative immunohistochemical staining images of SIRT1; (B) Quantitative analysis of the images showed XNJ increased the SIRT1 expression compared with I/R group; (C) Quantitative analysis of the images showed XNJ increased the SIRT1 expression relative to I/R group. (D) The SIRT1 activity was determined using a SIRT1 fluorometric assay kit. Data are represented as mean ± S.E.M. (*p < 0.05 vs. Sham; **p < 0.01 vs. Sham; ***p < 0.001 vs. Sham; #p < 0.05 vs. I/R; ##p < 0.01 vs. I/R; ###p < 0.001 vs. I/R). n = 4 in each group.
the XNJ-induced suppression of phosphorylated NF-κB/p65, adhesive factors (ICAM-1 and VCAM-1), and inflammatory cytokines (TNF-α, IL-1β, IL-6) (Figure 5). These results show that XNJ repressed cerebral I/R-induced inflammation via the SIRT1-autophagy pathway.

**XNJ induced the inhibition monocyte/macrophage extent in cerebral I/R injury rats was dependent on SIRT1**

Next, the role of SIRT1 on XNJ-induced inflammation inhibition was examined more intuitively at the morphological level. Macrophages are typically activated with the consequential release of proinflammatory cytokines post stroke. They have limited lifespan in the brain and are not present in large numbers under normal homeostatic conditions (Esiri and McGee 1986; Fischer-Smith et al. 2004). To explore the effect of XNJ on macrophage activation under I/R condition, we stained sections with anti-CD68, which detects resident monocyte/macrophages (Fischer-Smith et al. 2004). Strikingly, compared with sham, I/R obviously increased the number of monocyte/macrophages (CD68-positive cells) in the white matter of brain tissue. In addition, treatment with XNJ observably diminished I/R-induced monocyte/macrophage activation (Figure 6(A,B)), which was partly reversed by EX527, suggesting that XNJ-repressed macrophage activation is mediated by SIRT1.

**XNJ enhanced SIRT1 expression in OGD-cultured HBMECs**

To test whether XNJ affects SIRT1 in OGD-cultured HBMECs, western blot analyses were first conducted. XNJ treatment reversed OGD-induced SIRT1 repression in HBMECs (Figure 7(A)). Immunofluorescence assays were also performed to detect SIRT1 expression (Figure 7(B)) and showed that the fluorescence intensity of SIRT1 was increased by XNJ. Collectively, the data indicate that XNJ enhanced SIRT1 activity.

**SIRT1 mediates XNJ-regulated inhibition of the inflammatory response in OGD-cultured HBMECs**

To investigate the role of SIRT1 in the regulation of inflammation in XNJ-treated OGD-cultured HBMECs, the specific SIRT1 inhibitor EX527 was used to block SIRT1 activity. We first performed western blots and immunofluorescence assays to detect p65 phosphorylation and translocation, respectively. Western blotting indicated that the OGD-induced augmentation of p65 phosphorylation was markedly attenuated after XNJ pre-treatment; however, this effect was reversed by EX527 (Figure 8(A)). Consistently, immunofluorescence assays showed that p65 was in the cytoplasm in an inactive state in the control group, and XNJ reversed OGD-induced nuclear transfer of p65. Remarkably, most p65 translocated to the nucleus with EX527 treatment (Figure 8(B)). OGD inhibited IκBα expression, and EX527 attenuated the up-regulation effect of XNJ on IκBα (Figure 8(C)).

Finally, we detected the levels of adhesive factors and found that...
Figure 6. EX527 dramatically blocked the effects of XNJ on the repression of macrophage activation. (A) Representative immunohistochemical staining images of macrophage cells; (B) Quantification of macrophage/microglia cells. (\( ^{**} p < 0.01 \) vs. sham; \( ^{###} p < 0.001 \) vs. I/R; \( ^{##} p < 0.01 \) vs. I/R + XNJ). \( n = 4 \) in each group.

Figure 7. Effects of XNJ on SIRT1 expression in OGD-cultured HBMECs. (A) The protein expression of SIRT1 was analyzed by western blot; (B) SIRT1 was up-regulated in the HBMECs exposed to OGD pre-treated with XNJ by immunofluorescent staining. Positive stainings as shown in green. Scale bars: 50 \( \mu \)m. Data are represented as mean \( \pm \) S.E.M. (\( ^{**} p < 0.001 \) vs. CT; \( ^{#} p < 0.05 \) vs. OGD; \( ^{###} p < 0.001 \) vs. OGD). \( n = 4 \) in each group.
that ICAM-1 and VCAM-1 were obviously increased under OGD, while EX527 obviously reversed the repression of ICAM-1 and VCAM-1 induced by XNJ (Figure 8(D,E)). These results provide in vitro evidence that XNJ repressed inflammation via the SIRT1 pathway.

**Discussion**

In the present study, we established in vivo and in vitro models to evaluate the protective effects of XNJ against cerebral I/R injury. The results provide new evidence that XNJ can alleviate the cerebral I/R-induced inflammatory response and increase SIRT1 expression. Importantly, SIRT1 inhibition reversed the inhibitory effect of XNJ on inflammation. This suggests that XNJ treatment exerted neuroprotective effects by inhibiting inflammation via the SIRT1 pathway.

Inflammation reflects activation of the immune system and is thought to be a major contributor to cerebral I/R injury. It is characterized by the accumulation of inflammatory cells and the release of cytokines (TNF-α, IL-6, and IL-1β) (Amantea et al. 2009; Jin et al. 2010). NF-κB is an important transcription factor responsible for proinflammatory activation of microglia and the transcription of pro-inflammatory genes. Cerebral I/R injury causes inflammatory responses due to increased production of these cytokines. Other studies suggested that inhibition of cytokine production or function was important for controlling inflammation. Our results show that XNJ significantly attenuated morphological changes and reduced NF-κB/p65 activity and cytokine levels.

To further explore the mechanisms of XNJ-mediated suppression of inflammation, we investigated the role of SIRT1, which is a vital regulator of inflammatory processes. Zhu et al. (2019) showed that alleviation of cerebral inflammation by SIRT1 signalling was involved in butein attenuating sepsis-induced brain injury. Importantly, mounting evidence suggests that SIRT1 is an important neuroprotective molecule that participates in relieving cerebral I/R injury (He et al. 2017; Meng et al. 2017). Consistent with previous studies, we demonstrated that XNJ enhanced SIRT1 expression and blunted the inflammatory response. Notably, SIRT1 inhibition with EX527 dramatically reversed the repression of inflammation induced by XNJ. Based on these results, we deduced that XNJ modulation of inflammation is dependent on SIRT1 expression.

HBMECs are the first target of stroke and play a key role in brain vascular repair and maintenance, which are both impeded in stroke (Li et al. 2017). We established an OGD-cultured HBMEC model to mimic cerebral I/R injury in vitro and further explore how XNJ protects against I/R cerebral injury. We previously reported that XNJ could prevent OGD-induced apoptosis in HBMECs (Zhang et al. 2018); however, little is known about the effect of XNJ on inflammation in HBMECs. Here we demonstrated that OGD reduced SIRT1 level, resulting in up-regulation of NF-κB/p65 and leading to increased production of adhesion molecules. XNJ treatment reversed the expression of SIRT1 and inhibited inflammation in the OGD-cultured cells. Importantly, EX527 reversed the effect of XNJ on inflammation.

The results provide new evidence to guide future research on the use of XNJ for treating stroke. The rat MCAO model mimics human ischaemic disease and can be used to further explore the

**Figure 8.** SIRT1 regulated the remission of XNJ on inflammation in HBMECs. (A) EX527 markedly enhanced NF-κB/p65 activity relative to XNJ groups. (B) NF-κB/p65 translocation from cytoplasm to nucleus was detected. (C) IkBα expression was detected by western blot. (D) Adhesion molecules VCAM-1 and ICAM-1 were detected by western blot. Scale bars: 50 μm. Data are represented as mean ± S.E.M. (***p < 0.001 vs. CTL; ###p < 0.001 vs. OGD; ##p < 0.01 vs. OGD; #p < 0.05 vs. OGD + XNJ; #p < 0.01 vs. OGD + XNJ; ###p < 0.001 vs. OGD + XNJ). n = 4 in each group.
mechanism of XNJ, which will provide a firmer theoretical basis for the clinical application of XNJ.

Conclusions

XNJ treatment dramatically mitigated cerebral I/R injury in rats, which was modulated partially by blocking the inflammatory response via the SIRT1 pathway. Nevertheless, some limitations exist in our study, and we did not identify the specific pathological mechanisms of cerebral I/R injury. Further research on the mechanism of XNJ for the treatment of cerebral ischaemia is still needed.

Disclosure statement

The authors declare that there are no financial conflicts of interest in regard to this work.

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