The Neuroprotective Effects of Resveratrol Preconditioning in Transient Global Cerebral Ischemia–Reperfusion in Mice

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ABSTRACT

AIM: This study was designed to elucidate the neuroprotective effect of resveratrol in a mouse model of bilateral common carotid artery occlusion (BCCAO).

MATERIAL and METHODS: Sixty male C57BL/6 mice, weighing 20–24 g, were used in our experiments. The mice were randomly assigned into three groups: control group, BCCAO group and BCCAO+Resveratrol group. Neurological score was assessed 24h, 48h, 72h after BCCAO, respectively. Hematoxylin and eosin staining, NeuN and TUNEL were performed to detect the neuronal death and survival. The expression of Bcl-2, Bax, caspase-3, and cleaved caspase-3 were also detected to assess the anti-apoptotic effect of resveratrol by Western Blot.

RESULTS: Resveratrol significantly improved neurological score in BCCAO mice. Besides, it attenuates neuronal apoptosis via increasing the expression of Bcl-2 and decreasing the expression of Bax, caspase-3, and cleaved caspase-3. Resveratrol promotes neuronal survival in mice subjected to BCCAO.

CONCLUSION: Resveratrol is beneficial in the model of BCCAO, which is associated with its anti-apoptotic effect.

KEYWORDS: Resveratrol, Apoptosis, Global cerebral ischemia

INTRODUCTION

Ischemic stroke is caused by a transient or permanent occlusion in cerebral vessels. It causes neuronal death and associated behavioral deficits, including sensorimotor dysfunction, spatial orientation disorder, and learning and memory impairments (1, 13, 22). Stroke may induce a series of neurological disorders, such as oxidative stress, blood-brain barrier dysfunction, neuronal apoptosis and inflammation (8).

Resveratrol is a polyphenol found in high amounts in grapes, berries, peanuts, and medicinal plants such as Polygonum cuspidatum (2). It is produced by plants to protect themselves against stress, excessive sunlight, ultraviolet radiation, inflammation, and fungi (27). It was reported to be the solution to the “French Paradox”, a term used to describe the observation that the French population had a very low incidence of cardiovascular disease despite a high consumption of wine and saturated fat (17). Recently, resveratrol has received widespread attention for its potential use as a therapeutic agent in the prevention and treatment of numerous diseases, such as hypertension (10), coronary artery disease (21) and diabetes (30). Previous studies have suggested that resveratrol exhibits anti-inflammatory, anti-oxidant, and anti-carcinogenic properties (3, 6, 14, 28, 33).

This study aims to investigate whether resveratrol had a neuroprotective effect on ischemic neurons in a bilateral common carotid artery occlusion model. If so, we also wanted to find out whether it exhibited anti-apoptotic effect in this ischemic model.
MATERIAL and METHODS

Animals
Sixty C57BL/6 mice, weighing 20–24 g, were used in our experiments and they were provided by the Experimental Animal Center of the Shandong University. Mice were kept in a controlled-temperature room with a 12h/12h light/dark cycle. All the mice were allowed free access to food and tap water.

Establishment of Global Cerebral Ischemia
Bilateral common carotid artery occlusion (BCCAO) was used to trigger global cerebral ischemia. Mice were anesthetized with 3% isoflurane. After induction, the isoflurane concentration was maintained at 1.5%. A midline incision was made between the neck and sternum and the trachea was exposed. Then both the right and left common carotid arteries were carefully separated. Cerebral ischemia was induced by clamping both of the arteries with two miniature artery clips. After 20 minutes of cerebral ischemia, the clips were removed to allow for the reperfusion of blood through the carotid arteries. Sham-operated mice underwent the same surgical procedure without artery occlusion. During the surgery, the body temperature was monitored with a temperature probe and maintained at 37.0–37.5 °C using a heating pad.

Drug Administration
For the BCCAO+Resveratrol group, Resveratrol (dissolved in 2% dimethyl sulfoxide (DMSO)) was administered at a dose of 30 mg/kg i.p. for 7 consecutive days before surgery. Mice in the sham group and BCCAO group were injected solely with an equal concentration and amount of DMSO.

Neurological Tests
The treated mice were left to recover for 24 h before subsequent tests. The motor deficits of mice were tested with some modifications. Briefly, mice were placed on a 10- to 20-cm screen (grid size 0.2×0.2 cm) that could rotate from 0° (horizontal) to 90° (vertical). Mice were placed on the screen, which was in a horizontal position at first, and the screen was then rotated into the vertical position. The duration of each mouse on the vertical screen was recorded. The windows software (SPSS Inc., Chicago, IL). All values, except for total motor scores, are presented as the means ± the SDs and were analyzed using a one-way analysis of variance. Between-group differences were detected based on post hoc Student–Newman–Keuls tests. The total motor scores are expressed as the medians and were analyzed using the Kruskal–Wallis test. Values of P<0.05 were considered to be statistically significant.

Statistical Analysis

Hematoxylin and Eosin (H&E) Staining
Neuronal damage was assessed using H&E staining. Three days after the induction of ischemia, animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and transcardially perfused with 4% phosphate-buffered paraformaldehyde after a flush with 0.1 M phosphate-buffered saline. Brains were removed, and post-fixed at 4°C in 4% paraformaldehyde overnight and then cut in a freezing microtome. Six consecutive sections (12 μm) taken backward from the optic chiasm and that included the dorsal hippocampus were stained with H&E. The pyramidal neurons of the CA1 region were evaluated.
RESULTS

Neurological Score

In the Figure 1A-C, the neurological score in BCCAO group decreased dramatically compared with that in sham group ($P<0.05$) at 24h, 48h and 72h after the induction of ischemia. Resveratrol pre-treatment ameliorated the injury and the neurological score was elevated.

H&E Staining

Three days after reperfusion, the number of viable neurons in the CA1 region was dramatically decreased. Resveratrol pre-treatment significantly reduced the neuronal degeneration in the CA1 region compared with that in the sham group (Figure 2A-D).

NeuN and TUNEL

As shown in the Figure 3, ischemia triggered a significant loss of neurons in the CA1 region as indicated by the decrease of NeuN-positive cells compared with the sham group. However, the survival of neurons was dramatically increased when resveratrol was given. Besides, ischemia induced a dramatic neuronal apoptosis in comparison with the sham group.
However, resveratrol pre-treatment attenuated the apoptosis of neurons as indicated by the decrease of TUNEL-positive neurons in the CA1 region.

**Western Blot**

The expression of the anti-apoptotic protein Bcl-2 and the pro-apoptotic protein Bax was displayed in Figure 4 A,B. BCCAO lowered Bcl-2 expression and increased Bax expression in comparison with the sham group. On the contrary, in comparison with BCCAO group, pre-treatment with resveratrol elevated Bcl-2 expression and decreased Bax expression. In addition, in comparison with the sham group, BCCAO-induced caspase-3 and cleaved caspase-3 up-regulation was markedly abolished by resveratrol pre-treatment (Figure 4 C,D).

**DISCUSSION**

The results of this study demonstrate that resveratrol preconditioning has a neuroprotective effect in cerebral ischemia–reperfusion in mice. Resveratrol preconditioning significantly improved the TMS and reduced neuronal death after cerebral ischemia–reperfusion. We found that resveratrol preconditioning induced an anti-apoptotic effect, which is shown by its ability to increase Bcl-2 expression and to decrease the expression of Bax, cleaved caspase-3 and caspase-3.

Pyramidal neurons of the CA1 region are particularly vulnerable to ischemic injury. This region undergoes delayed neuronal death, often reported as apoptosis united with DNA fragmentation (20). It has been suggested that resveratrol can cross the blood-brain barrier and protect against global
cerebral ischemic injury and inhibit glial cell activation (32). In a rat model of permanent bilateral common carotid artery occlusion, resveratrol improved learning and memory abilities, which are associated with its anti-oxidant effects (18). Nitric oxide was reported to play a critical role in the neuroprotective effects of resveratrol in focal cerebral ischemia (12, 28). In addition, resveratrol protects rat brain from cerebral ischemic injury via a sirtuin 1-uncoupling protein 2 and TRPC6/CREB pathways (7, 16). Resveratrol also regulates the expression of various proteins associated with oxidative stress and energy metabolism in focal cerebral ischemia (24). Furthermore, resveratrol can enhance the neuroprotection when co-administered with lipoic acid in cerebral ischemia (24). In the present study, we first established the model of BCCAO, and then we found that resveratrol pretreatment could protect the brain from ischemic injury, which is associated with its anti-apoptotic effect.

Neuronal apoptosis is a key pathological process of ischemic stroke (4, 9). Several experimental studies have demonstrated that the inhibition of apoptosis reduces ischemic injury (23). Though two pathways of apoptosis (the extrinsic and the intrinsic) have been demonstrated (5), the final phase of apoptosis execution that includes activation of executioner caspases (e.g., caspase 3) is shared by both these pathways (29). Interactions between the pro-apoptotic Bax and anti-apoptotic Bcl-2 family proteins on the mitochondria are believed to play an important role in cell survival (31). The anti-apoptotic protein Bcl-2 is capable of preventing cytochrome c release, which is an activator of apoptosis, and promotes cell survival, while the pro-apoptotic protein (Bax) promotes cell death (19). Resveratrol has been reported to up-regulate Bcl-2 expression in a rat model of focal cerebral ischemia (15). So in the present study, we detected the expression of Bax and Bcl-2 using Western blot, and the results suggest that resveratrol increases Bcl-2 expression and decreases Bax expression, indicating that the protective effect against cerebral ischemia is related to inhibiting apoptosis. Moreover, we also assayed the expression of caspase-3 and cleaved caspase-3, and the results suggest that resveratrol pretreatment lowers the expression of the two proteins, indicating that resveratrol protects the brain from ischemia via inhibiting the final phase of apoptosis.

**CONCLUSION**

Resveratrol provides significant neuroprotection in mice subjected to global cerebral ischemia via inhibiting neuronal apoptosis (increasing Bcl-2 expression and decreasing the expressions of Bax, caspase-3 and cleaved caspase-3).

**REFERENCES**


