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Original article

Magnolol attenuates the inflammation and apoptosis through the activation of SIRT1 in experimental stroke rats

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A B S T R A C T

Background: Silent information regulator 1 (SIRT1), a histone deacetylase, plays a protective role in ischemic brain injury. Previous studies have shown that magnolol has a beneficial effect on ischemic stroke; however, the role of SIRT1 in the protective effect of magnolol against cerebral ischemia has not been investigated.
Methods: We used a middle cerebral artery occlusion model of stroke in rats. Before stroke induction, the rats received intraperitoneal injections of magnolol with or without the SIRT1 inhibitor, EX527. Brain water content, neurological score, and infarct volume were measured. Moreover, the levels of the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured. Western blot analysis was performed to detect Ac-FOXO1, SIRT1, bax, and Bcl-2 expression.
Results: Magnolol exerted a beneficial effect on cerebral ischemia, as indicated by reduced brain edema, decreased infarct volume, and improved neurological score. Magnolol had an anti-inflammatory effect mediated by a decrease in the expression of IL-1β and TNF-α in the brain tissue. Additionally, magnolol down-regulated bax and Ac-FOXO1 expression and up-regulated Bcl-2 and SIRT1 expression. This effect of magnolol was abolished by EX527 treatment.
Conclusion: In conclusion, our data clearly indicate that magnolol modulates brain injury caused by ischemic stroke by inhibiting inflammatory cytokines and apoptosis through SIRT1 activation.

Introduction

Ischemic stroke is a leading cause of morbidity and mortality worldwide. The middle cerebral artery (MCA) and cerebral vessels are involved in ischemic stroke. Reduced blood flow caused by occlusion of the MCA and cerebral vessels triggers a series of pathophysiological events, such as oxidative stress, glutamate excitotoxicity, mitochondrial dysfunction, apoptosis, inflammation, and calcium overload. Although thrombolytic agents are generally used to treat ischemic stroke, the drugs have a narrow therapeutic window and their safety has been questioned [1]. Given these limitations, there is an urgent need for the development of novel agents with greater therapeutic efficacy for ischemic stroke [1–3].

Silent information regulator 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase. SIRT1 expression decreases with aging and is believed to play a key role in the longevity effects elicited by calorie restriction. Forkhead box O (FOXO) acts through SIRT1 to activate the synthesis of antioxidants, such as catalase and superoxide dismutase, which induces cellular resistance against oxidative stress. Furthermore, SIRT1 has been shown to play an important role in neuroprotection against cerebral ischemia [4–6]. SIRT1 mediates hyperbaric oxygen preconditioning-induced ischemia following cerebral ischemia reperfusion injury. Furthermore, SIRT1 plays a critical role in the protection against cerebral ischemia. Previous studies have shown that magnolol protects against ischemic stroke [7]. Moreover, magnolol has a protective effect against chemically induced hepatic injury *via* SIRT1-mediated deacetylation of p53 [8]. However, the role of SIRT1 in the protective effect of magnolol against cerebral ischemia is not clear.

Material and methods

Experimental subjects

The experimental subjects were Sprague Dawley rats (200–250 g) purchased from the department animal facility. The animals

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were individually housed under standard laboratory conditions with a 12/12 h dark/light cycle, temperature of 20 ± 5 °C, and 60 ± 5% humidity. The animals had free access to food and water. All experimental procedures were performed in accordance with the Institutional Guide for the Care and Use of Laboratory Animals.

Middle cerebral artery occlusion procedure

MCA occlusion (MCAO) was performed using a modified version of a previously reported procedure [9]. The rats were anesthetized by the intraperitoneal (*ip*) injection of chloral hydrate (10%), and the right carotid artery was exposed and immediately washed. The middle cerebral artery was occluded by inserting a monofilament nylon suture with a heat-rounded tip into the internal carotid artery, which was superior to the MCA, until it blocked the origin of the MCA [10]. After the induction of ischemia (30 min), reperfusion was initiated by withdrawing the monofilament. The normal control rats were exposed to the same surgical procedure without MCAO. A heat lamp or heating pad was used to maintain the rectal temperature of all animals during the surgery.

Experimental protocol

To induce ischemia, intraperitoneal (*ip*) injections of magnolol (25 mg/kg) dissolved in normal saline were administered twice (immediately after the induction of ischemia and when reperfusion was initiated). The SIRT1 inhibitor (EX527) was dissolved in dimethyl sulfoxide (DMSO) and the final concentration was adjusted using normal saline (the final concentration of DMSO was less than 2%). Before MCAO, the rats received i.p. injections of EX527 (5 mg/kg), and the same amount of vehicle was injected in the animals four times every 2 days. The rats were randomly divided into the following groups of 10 rats each: Group I: normal control, Group II: MCAO control, Group III: MCAO + magnolol (25 mg/kg), Group IV: MCAO + magnolol (25 mg/kg) + EX527, and Group V: MCAO + EX527.

Assessment of neurological deficits

Neurological deficits were assessed 24 h after the induction of ischemia by observers blinded to the MCAO treatment groups. Neurological deficits were scored as follows: 0: normal (no motor deficits), 1: mild (forelimb weakness and torso turning to the ipsilateral side when held by the tail), 2: moderate (circling to the contralateral side but normal posture at rest), 3: severe (unable to bear weight on the affected side at rest), and 4: critical (barrel rolling or no spontaneous locomotor activity) [11].

Brain water content

To estimate brain water content, wet brain tissue was weighed and quantified, and the white and gray regions of the brains were desiccated for 48 h at 105 °C until the weight of the brain was constant [12]. The weight of the 2,3,5-triphenyltetrazolium chloride (TTC)-stained brains was calculated as the total weight of the white and gray desiccated brain tissue, and the water content in both brain regions was estimated using the following formula (Eq. 1):

$$Water\ content(\%) = \frac{(wet\ weight - dry\ weight)}{wet\ weight} \times 100.$$

Estimation of the infarct volume

TTC was used to assess infarct volume 24 h after reperfusion. All rats were euthanized and brain sections were immediately removed, cut into five 3-mm-thick coronal sections, and stained with TTC (2%) solution at room temperature (37 °C) for 20 min, followed by fixation in 4% formaldehyde [13]. The tissue sections were mounted on slides and images were captured using a digital camera. Lesion volume was calculated by summing the areas measured and multiplying by the slice thickness. The percentage hemisphere lesion volume (%HLV) was estimated using the following formula (see below Eq. 2).

$$\%HLV \frac{([total\ infarct\ volume - (volume\ of\ intarct\ ipsilateral\ hemisphere - volume\ of\ intarct\ contralateral\ hemisphere)]}{contralateral\ hemispere\ volume} X100 \quad (2)$$

Inflammatory cytokine measurement

Levels of the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) were measured in the brain tissue using enzyme-linked immunosorbent assay (ELISA) kits, in accordance with the procedure recommended by the manufacturer [14].

Western blot analysis

The ischemic penumbra of the cerebral cortex was obtained for Western blot analysis. The tissue samples were lysed in ice-cold lysis buffer (1 mM EDTA, 50 mM Tris–HCl, 1 mM EGTA, 0.1% 2-mercaptoethanol, 0.5 mM Na_3VO_4, 50 mM NaF, 1% Triton X-100, 10 mM sodium β-glyceropyrophosphate, 5 mM sodium pyrophosphate, 0.1 mM phenylmethanesulfonyl fluoride, and a mixture of protease inhibitors) for 10 min. Following lysis, the tissue samples were centrifuged at 12,000*g* for 15 min, and the supernatant was collected and boiled. The bicinchoninic acid method was used to determine the protein concentration in the extracts. The protein (50 μg) was resolved on SDS polyacrylamide gel membranes (8–12%), and transferred to nitrocellulose membranes. The nitrocellulose membrane was blocked with nonfat milk (5%) in Tris-buffered saline containing Tween (TBST) and then incubated with antibodies against Bcl2, β-actin, bax, Ac-FOXO1, and SIRT1 overnight at 4 °C. The tissue was then incubated with secondary antibodies for 120 min at room temperature, rewashed with TBST, and the protein bands were detected using the Bio-Rad imaging system (Bio-Rad, Hercules, CA, USA).

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). An analysis of variance followed by Dunnett's test was used to determine significant differences. The statistical tests were conducted using the Statistical Package for the Social Sciences ver. 12 (SPSS Inc., Chicago, IL, USA), *p*-values < 0.05 were deemed to indicate statistical significance.