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Icariin protects against intestinal ischemia–reperfusion injury

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ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form
14 August 2014
Accepted 2 October 2014
Available online 8 October 2014

Keywords:
Intestinal ischemia–reperfusion
SIRT1
Icariin
FOXO3
Oxidative stress
Apoptosis

ABSTRACT

Background: This study investigated the role of Sirtuin 1 (SIRT1)/forkhead box O3 (FOXO3) pathway, and a possible protective function for Icariin (ICA), in intestinal ischemia–reperfusion (I/R) injury and hypoxia–reoxygenation (H/R) injury.

Materials and methods: Male Sprague–Dawley rats were pretreated with different doses of ICA (30 and 60 mg/kg) or olive oil as control 1 h before intestinal I/R. Caco-2 cells were pretreated with different concentrations of ICA (25, 50, and 100 μg/mL) and then subjected to H/R-induced injury.

Results: The *in vivo* results demonstrated that ICA pretreatment significantly improved I/R-induced tissue damage and decreased serum tumor necrosis factor α and interleukin-6 levels. Changes of manganese superoxide dismutase, Bcl-2, and Bim were also reversed by ICA, and apoptosis was reduced. Importantly, the protective effects of ICA were positively associated with SIRT1 activation. Increased SIRT1 expression, as well as decreased acetylated FOXO3 expression, was observed in Caco-2 cells pretreated with ICA. Additionally, the protective effects of ICA were abrogated in the presence of SIRT1 inhibitor nicotinamide. This suggests that ICA exerts a protective effect upon H/R injury through activation of SIRT1/FOXO3 signaling pathway. Accordingly, the SIRT1 activator resveratrol achieved a similar protective effect as ICA on H/R injury, whereas cellular damage resulting from H/R was exacerbated by SIRT1 knockdown and nicotinamide.

Conclusions: SIRT1, activated by ICA, protects intestinal epithelial cells from I/R injury by inducing FOXO3 deacetylation both *in vivo* and in *vitro* These findings suggest that the SIRT1/FOXO3 pathway can be a target for therapeutic approaches intended to minimize injury resulting from intestinal dysfunction.

1. Introduction

Intestinal ischemia–reperfusion (I/R) injury is a pathophysiological process typically associated with intestinal and mesenteric vascular dysfunction, which can also occur as a result of surgery, organ transplantation, and trauma. Intestinal I/R is the initiating event in systemic inflammatory response syndrome and multiple organ dysfunction syndrome, associated with significant morbidity and mortality [1,2]. Thus, it is important to understand the mechanisms that

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http://dx.doi.org/10.1016/j.jss.2014.10.004

mediate the cellular response to injury, and exploit these to mitigate damage to tissue and to the organism.

Sirtuin 1 (SIRT1) is a member of the highly conserved nicotinamide adenine dinucleotide-dependent class III histone deacetylases [3,4], which is involved in multiple cellular processes, including DNA damage response, cell cycle regulation, apoptosis, and cellular longevity [5]. Recently, SIRT1 has been shown to play an important role in preventing oxidative damage resulting from myocardial hypoxia–reoxygenation (H/R), acute kidney injury, and hepatic stress [6–10]. During myocardial I/R injury, SIRT1 messenger RNA (mRNA) and protein levels decreased significantly; moreover, mice deficient in cardiac-specific SIRT1 exhibited a greater myocardial infarct size than wild-type mice after I/R [11], indicating that SIRT1 may have protective effects against I/R injury.

The forkhead box O (FOXO) family of transcription factors is involved in cell-cycle regulation, DNA repair, and oxidative stress resistance and is also known to have antiapoptotic function [12,13]. FOXO3 has been shown to promote resistance to oxidative stress in cells under hypoxic and/or ischemic conditions, mainly by activating the expression of its target gene superoxide dismutase, which is a scavenger of oxygen free radicals [14]. FOXO3 is also involved in the regulation of apoptosis [15]. Recent reports indicate that SIRT1 can directly modulate FOXO3 deacetylation and activities under conditions of cellular stress [16,17]. However, the function of FOXO3 in intestinal I/R injury, and its relationship to SIRT1, has yet to be determined.

Icariin (ICA), a flavonoid extracted from the traditional Chinese herb *Epimedium brevicornum* Maxim, is known to have anti-inflammatory, antioxidative, and antiapoptotic properties [18–20]. Recently, a neuroprotective function has been observed for ICA on injury induced by oxygen and glucose deprivation, and this effect was associated with an upregulation of SIRT1 [21–23].

We hypothesized that ICA could protect against oxidative stress and apoptosis induced by intestinal I/R, and these beneficial effects may be associated with SIRT1/FOXO3 signaling activation. To test this hypothesis, we determined whether ICA exerts protective effects in a rat model of intestinal I/R injury and in Caco-2 cells H/R injury and examined SIRT1/FOXO3 signaling pathway variation both *in vivo* and *in vitro*.

2. Materials and methods

2.1. Drugs and reagents

ICA (98% pure), purchased from Shanghai Winherb Medical Science Co, Ltd (Shanghai, China), was dissolved in olive oil (3 and 6 mg/mL) and gavaged before intestinal I/R (at the dose of 10 mL/kg). The rats in the sham and I/R groups were treated with an equal volume of olive oil. The dose of ICA administration was determined from a previous study with modification from our preliminary experiments [23,24]. In cell culture experiments, ICA was dissolved in 0.1% dimethyl sulfoxide at three concentrations (25, 50, and 100 μg/mL), and the cells were treated with ICA for 6 h before exposure to H/R environment. An equal volume of 0.1% dimethyl sulfoxide was applied as the control. Nicotinamide (NAM, 98% pure) was purchased from Shanghai Source Leaf Biological Technology Co, Ltd (Shanghai, China) and was dissolved in saline.

2.2. Animals and experimental groups

Male Sprague–Dawley rats weighing 180–220 g were obtained from the Animal Center of Dalian Medical University (Dalian, China), housed under specific pathogen-free conditions and provided with standard laboratory chow and water. The rats were fasted overnight with free access to water before operation.

The rats were divided into five experimental groups randomly, with eight rats in each of the following: (1) sham-operated group (sham); (2) intestinal I/R group (I/R); (3) sham + ICA (60 mg/kg), the rats were pretreated with ICA at a dose of 60 mg/kg intragastrically for three consecutive days, and then surgery was performed as that in the sham group; (4) I/R + ICA (30 mg/kg), the rats were pretreated with ICA at a dose of 30 mg/kg for 3 d, and then surgery was performed as that in the I/R group; and (5) I/R + ICA (60 mg/kg), the rats were pretreated with ICA at a dose of 60 mg/kg, and then surgery was performed as that in the I/R group.

The intestinal I/R model was established according to previous standardized procedures with modification from preliminary experiments [25]. The rats in the sham group underwent surgical preparation including isolation of the superior mesenteric artery without occlusion. The rats in the I/R group were subjected to 1 h intestinal ischemia and 2 h reperfusion after the superior mesenteric artery was isolated and occluded by an atraumatic microvascular clamp. All animals were euthanized at the end of the reperfusion, and tissues and blood samples were harvested for analysis. All procedures were conducted according to the institutional animal care guidelines and were approved by the institutional ethics committee.

2.3. Intestine morphologic assessment

The isolated intestine was fixed in 4% paraformaldehyde for 24 h, embedded in paraffin, sliced into 4-μm sections, and stained with hematoxylin-eosin according to standard procedures. Pathologic score of the intestine damage was evaluated according to previous reports [26].

2.4. Measurement of serum tumor necrosis factor-α and interleukin-6 by enzyme-linked immunosorbent assay

The blood samples were harvested from the abdominal aorta and allowed to coagulate for 30 min at room temperature. Serum was isolated after centrifugation at 2500 rpm for 15 min. The levels of serum tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 were measured with enzyme-linked immunosorbent assay kits (ENGTON bio-engineering Co, Ltd, Shanghai, China), according to the manufacturer's protocols.