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Osthole prevents intestinal ischemia-reperfusion–induced lung injury in a rodent model

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ABSTRACT

Background: Intestinal ischemia–reperfusion (II/R) is associated with high morbidity and mortality. The aim of this study was to investigate the effects of osthole on lung injury and mortality induced by II/R.

Methods: A rat model of II/R was induced by clamping the superior mesenteric artery for 90 min followed by reperfusion for 240 min. Osthole was administrated intraperitoneally at 30 min before intestinal ischemia (10 or 50 mg/kg). The survival rate and mean arterial pressure were observed. Blood samples were obtained for blood gas analyses. Lung injury was assessed by the histopathologic changes (hematoxylin and eosin staining), lung wet-to-dry weight ratio, and pulmonary permeability index. The levels of reactive oxygen species, malondialdehyde, interleukin 6, and tumor necrosis factor α, as well as the activities of superoxide dismutase and myeloperoxidase in lung were measured.

Results: The survival rate, ratio of arterial oxygen tension to fraction of inspired oxygen, and mean arterial pressure decreased significantly after II/R. Results also indicated that II/R-induced severe lung injury evidenced by increase in pathologic scores, lung wet-to-dry weight ratio, and pulmonary permeability index, which was accompanied by increases in the levels of pulmonary reactive oxygen species, malondialdehyde, interleukin 6, tumor necrosis factor α, and the pulmonary myeloperoxidase activity and a decrease in superoxide dismutase activity. Osthole could significantly ameliorate lung injury and improve the previously mentioned variables.

Conclusions: These findings indicated that osthole could attenuate the lung injury induced by II/R in rats, at least in part, by inhibiting inflammatory response and oxidative stress.

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1. Introduction

Intestinal ischemia–reperfusion (II/R) injury is a common but grave condition resulting from acute mesenteric ischemia, hemorrhagic, traumatic or septic shock, or severe burns and some surgical procedures [1]. It is well known that II/R not only causes intestinal injury [2] but also leads to severe destruction of remote organs and even multiple organ dysfunction [3–5]. Therefore, it presents a very high mortality and morbidity in the critical care setting. Of these remote organ injuries induced by II/R, the lung is one of the most vulnerable organs and lung injury has been well-characterized as an acute inflammation with sequestration of leukocytes and their enzymatic products in lung tissue, increased microvascular permeability, perivascular and interstitial edema, and pulmonary edema [6,7].

The mechanisms of remote organ injuries induced by II/R are very complex. The underlying mechanisms of lung injury are related to overt generation of reactive oxygen species (ROS) and proinflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α caused by II/R [1,8]. Therefore, anti-inflammatory and antioxidative agents are urgently required to ameliorate II/R-induced lung injury.

Cnidium monnieri (L.) Cusson is a plant, and its dried fruit called "She-Chuang-Zi" in China has been used as a traditional remedy to treat male sexual dysfunction for several hundred years [9]. The extracts of *C monnieri* (L.) Cusson contain several ingredients, including bergapten, isopimpinellin, xanthotoxin, imperatorin, and osthole [10]. Osthole (7-methoxy-8-isopentenoxy-coumarin; molecular formula, $C_{15}H_{16}O_3$; molecular weight, 244.39 Da; chemical structure shown in Fig. 1), a natural coumarin derivative, can be isolated from several medicinal plants, such as *C monnieri* (L.) Cusson and *Peucedanum ostruthium* [9,11]. As the main bioactive constituent of *C monnieri* (L.) Cusson, osthole is a molecule monomer and it is used as a lead compound in new drug discovery area because of its unique structural modification. Interestingly, osthole has been widely used for treating gynecopathy and skin disease [12]. Of note, osthole has received considerable attention for its broad spectrum of pharmacologic activities, including anti-inflammatory [13] and antioxidative [14]. Recently, increasing data have demonstrated that osthole exerts protection against organic I/R injury, such as intestine [15], brain [14], spinal cord [16], and kidney [17]. Interestingly, recent studies have shown that osthole treatment attenuates lipopolysaccharide-induced acute lung injury in a murine model [18,19]. However, to date, the effects of osthole on lung injury induced by II/R remain unclear.

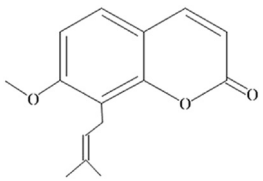

Fig. 1 – The chemical structure of osthole.

On the basis of the previously mentioned findings, we hypothesized that osthole can reverse II/R-induced lung injury. Therefore, the present study was undertaken to confirm the previously mentioned hypothesis and elucidate the mechanisms related to pulmonary oxidative stress, inflammation, and neutrophil sequestration in a rat model.

2. Experimental procedures

2.1. Animal model

Adult male Wistar rats, weighing 265–320 g, were provided by the Laboratory Animal Center of Luzhou Medical College, Luzhou, China. They were housed with five to a cage in a temperature-controlled room with alternating 12 h light–dark cycle and acclimated for a week before the study. All rats were fasted for 8 h but allowed water *ad libitum* before manipulations. The present study was approved by the Animal Care Committee of Luzhou Medical College, Luzhou, China. Additionally, animal care and handling were performed in accordance with the National Institutes of Health guidelines.

2.2. Drug preparation and administration

Osthole (purity >98%; Longquan High-Tech natural pharmaceutical Co Ltd, Chengdu, China) was dissolved in the vehicle (a 1:9 [vol/vol] mixture of Tween 80 and 0.9% sodium chloride). Furthermore, osthole was intraperitoneally administered at 30 min before intestinal ischemia according to the previous study [17].

2.3. Experimental protocol

Rats were allocated into one of four groups: (1) sham group plus vehicle (10 mL/kg, sham); (2) II/R plus vehicle (10 mL/kg, II/R); (3) II/R plus osthole (10 mg/kg, II/R + Ost 1); and (4) II/R plus osthole (50 mg/kg, II/R + Ost 2). An equal volume of vehicle (in the sham and II/R groups) or osthole (in the II/R + Ost 1 and II/R + Ost 2 groups) was administered intraperitoneally 30 min before intestinal ischemia.

All animals were anesthetized with pentobarbital sodium (30 mg/kg, intraperitoneally), and a polyethylene catheter was inserted into the right carotid artery to monitor the mean arterial pressure (MAP). After tracheal intubation, rats were ventilated with 95% oxygen using a small animal ventilator at a ventilatory rate of 80 breaths/min and tidal volume of 2.5 mL to maintain the arterial carbon dioxide tension between 35 and 45 mm Hg. II/R was established as in our previous studies [2,3]. In brief, the small intestine was exteriorized by midline laparotomy and the II/R injury was established by occluding the superior mesenteric artery (SMA) with a microvascular clip. After 90 min of ischemia, the SMA was reperfused. Ischemia and reperfusion were recognized by the existence of pulseless or pale color of the small intestine, the return of pulses, and the reestablishment of the pink color,