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Administration of SB239063, a potent p38 MAPK inhibitor, alleviates acute lung injury induced by intestinal ischemia reperfusion in rats associated with AQP4 downregulation

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ABSTRACT

Acute lung injury (ALI), induced by intestinal ischemia reperfusion (II/R) injury, is characterized by pulmonary edema and inflammation. Aquaporin 4 (AQP4), has been pointed out recently involving in edema development. Previous studies have shown that p38 mitogen activated protein kinase (MAPK) activation resulted in lung inflammation, while p38 MAPK inhibitor can alleviate the pathology injury of lung tissue. However, the regulated mechanism of p38 MAPK in ALI induced by II/R is unclear. In this study, we established II/R rats' model by clamping the superior mesenteric artery (SMA) and coeliac artery (CA) for 40 min and subsequent reperfusion for 16 h, 24 h, 48 h. Subsequently, SB239063, a specific inhibitor of the activity of p38 MAPK, was injected (10 mg/kg) intraperitoneally 60 min before the operation. The severity of ALI was determined by histology analysis (HE staining and ALI scoring) and lung edema (lung wet/dry weight ratio) assessment. Western blot (WB) was applied to detect the expression level of AQP4 and phosphorylated (P)-p38 MAPK, and the localization of AQP4 was detected by immunofluorescent staining (IF). We found that AQP4 could express in the lung tissue. II/R could significantly induce lung injury, confirmed by lung injury scores and lung wet/dry weight ratios. The level of P-p38 MAPK and AQP4 were largely up-regulated in lung tissues. Moreover, inhibition of p38 MAPK activity could effectively down-regulate AQP4 expression and diminish the severity of II/R-induced ALI. These novel findings suggest that inhibition of p38 MAPK function should be a potential strategy for the prevention or treatment of ALI, by targeting AQP4 in future clinic trial.

1. Introduction

Intestinal ischemia-reperfusion (II/R) injury is a serious clinical event which always occurs in some critical practices such as superior mesenteric artery (SMA) occlusion and thrombus, hemorrhagic shock, or small bowel transplantation [1,2]. II/R not only leads to severe intestine damage but also induces subsequent destruction of remote organs including liver, lung, and kidney as well [3,4]. Nowadays, acute lung injury (ALI) can be caused by many diseases, such as brain ischemia and II/R [5–7]. ALI induced by II/R is triggered by the release of proinflammatory cytokines and bacteria-derived endotoxins from the reperfused ischemic gut tissue [8–10]. When ALI occurs, inflammatory reaction will damage the lung endothelium, resulting in high permeability of the lung capillaries to fluid, which leads to clinical pulmonary edema [11]. Therefore, inflammation and pulmonary edema may be two important pathological characteristics for this ALI [11,12].

Previous studies have shown that p38 mitogen activated protein kinase (MAPK) signaling pathway is activated in response to multiple inflammatory signals, including inflammatory cytokines, oxidative stress, and growth factors [13]. In addition, p38 MAPK is expressed in different tissues and regulates activation of different kinases and phosphorylation of different substrates, causing diverse and often opposing effects

Abbreviations: ALI, acute lung injury; II/R, intestinal ischemia reperfusion; MAPK, mitogen activated protein kinase; AQP4, aquaporin 4; SMA, superior mesenteric artery; CA, coeliac artery; P, phosphorylated; WB, western blot; IF, immunofluorescent staining; NIH, National Institutes of Health; SD, Sprague-Dawley; IL-1β, interlukin-1β, IL-6, interlukin-6; TNF-alpha, tumor necrosis factor-alpha; AQPs, aquaporins; HE, hematoxylin-eosin; PVDF, polyvinylidene difluoride.

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[14,15]. Recently, the p38 MAPK signaling pathway has been known to play crucial roles in inflammatory responses in ALI [14,16]. Moreover, p38 MAPK signaling pathway was also involved in the cerebral ischemia/reperfusion injury, myocardial ischemia/reperfusion injury and ALI induced by II/R [17,18]. Additionally, p38 MAPK inhibitors could alleviate inflammation in some diseases such as LPS-induced innate immune responses in murine intestinal myofibroblasts [19], hyperlipidemia and rheumatoid arthritis [20,21]. For example, SB239063, an inhibitor of p38 MAPK, has been developed to inhibit not only LPS induced injury, by suppressing neutrophils and IL-6 expression in the skin in guinea pigs [22], but also ischemia induced expression of IL-1β or IL-6 in ALI from II/R [23,24]. Therefore, interference of p38 MAPK signal by regulating inflammatory cytokines may be available to the treatment of ALI induced by virus, toxic substances and other inflammatory diseases [25–27], while the other regulating mechanisms are not clear. In addition, although p38 MAPK acts as an important mediator of inflammatory lung injury, the regulating mechanism of p38 MAPK, especially in lung edema from ALI induced by II/R still remains to be elucidated.

Aquaporins (AQPs) are a family of small integral membrane proteins that contribute significantly to water homeostasis by regulating water transport in the body [28]. Previous studies have showed that AQPs play important roles in physiology and several disease pathways [28,29]. It has been well known that AQPs composed of several forms in lung: AQP1 is found in the peribronchiolar, alveolar endothelia and in the visceral pleura; AQP3 is in the trachea; AQP4 is in airway epithelia and in the trachea; and AQP5 is at the apical membrane of type I alveolar epithelial cells [30].

Cell-specific and expressional differences of AQPs in the lung and airways have provided indirect evidence to support their different roles [31]. Studies of epithelia ion and fluid transport across the distal pulmonary epithelia have provided novel concepts to find potential therapies for ALI [32,33]. Recently, it has been pointed that up-regulation of AQP4 expression could be an important determinant of the overall water content on the basis of its involvement in the formation and elimination of edema [34]. In humans, the expression of AQP4 was found in cases of cerebral ischemia, glial tumors, traumatic brain injury, infection, and inflammatory diseases of the central nervous system [35]. Recently, it has been demonstrated that the down-regulation of AQP4 was associated with the neuroprotection and cardioprotection against the injury in the brain or heart induced by ischemia/reperfusion [36–39]. Although AQP4 up-regulation is involved in water balance regulation in the brain in cases of cerebral edema resulted from brain trauma and cerebral ischemia/reperfusion [40,41], its role in the development and resolution of pulmonary edema induced by II/R remains controversy. Moreover, whether p38 MAPK inhibition could regulate AQP4 expression in intestinal ischemia induced lung injury is not clear.

In this study, we tested whether AQP4 was associated with ALI induced by II/R, and p38 MAPK inhibition could protect lung from injury associated with AQP4 regulation. Our crucial findings may contribute to the treatment of ALI in the future clinic trail.

2. Methods

2.1. Animals and grouping

Animal care and all experimental protocols were approved by the guidelines of the Institutional Medical Experimental Animal Care Committee of Sichuan University, West China Hospital, China. Guidelines for laboratory animal care and safety from National Institutes of Health (NIH) were also followed. Adult male Sprague-Dawley (SD) rats, weighing 200–220 g, were provided by the Experimental Animal Center of Sichuan University. Animals were housed in individual cages in the temperature at 21–25 °C and humidity of 45–50%, they were fasting diet 24 h with free access to water prior to operation. At 12 h prior to the operation, food was removed but with no limitation to water.

Table 1
Animal model preparation and sample used.

Groups	Model	Lung edema	HE	IF	WB
Sham	Sham	8	8	6	8
16 h	II/R	8	8	6	8
24 h	II/R	8	8	6	8
48 h	II/R	8	8	6	8

HE, hematoxylin and eosin stain; IF, immunofluorescence; WB, western blotting; II/R, intestinal ischemia/reperfusion, including reperfusion 16 h, 24 h, 48 h.

The rats were randomly divided into four groups as described in Table 1 and Table 2. Group I, rats served as sham-operated controls. Group II, rats as II/R group were subjected to 40 min ischemia and 16 h, 24 h, 48 h reperfusion, respectively. Group III, rats as inhibitor group were received SB239063 (10 mg/kg). Group IV, rats as control group were received equal normal saline.

2.2. Intestinal ischemia and reperfusion model

II/R was induced by superior mesenteric artery (SMA) and coeliac artery (CA) occlusion as previously described [42,43]. At first, rats were anesthetized intraperitoneally (i.p.) with 3.6% chloralhydrate (1 mL/100 g, SG1019, TongYao biological technology co., LTD, Shanghai, China) and fixed in a supine position. The SMA and CA were isolated via median abdominal incision and clamped with an atraumatic microvascular clip for 40 min, followed by 16 h, 24 h, 48 h intestinal reperfusion, then closed the abdomen. The II/R + SB239063 group was injected intraperitoneally with a specific inhibitor of p38 activity, SB239063 (10 mg/kg, Haoyuan Chemexpress biological technology co., LTD, Shanghai, China) 1 h before the operation and clamped SMA, CA 40 min, and reperfusion for 16 h [23]. Rats of the sham group was only submitted to isolate SMA and CA for 40 min, then closed the abdomen.

2.3. Observation of lung edema

At 16 h, 24 h, 48 h post reperfusion, the lungs were taken out and weighted for wet weight immediately. Then the dry weight of the lung was recorded after drying in the 90 °C oven for 24 h. Therefore, lung wet/dry weight ratio was calculated for lung edema detection.

2.4. Examination of lung injury

At 16 h, 24 h, 48 h following reperfusion, lung tissue samples were fixed in 4% paraformaldehyde in PBS and embedded in paraffin after dehydration. Then, sections were stained with hematoxylin-eosin (H&E) and observed under light microscopy to detect lung injury. The lung injury was scored as previously described [44], and the score scaled at 0 to 4 represents the severity of the lung injury. After evaluating, the total lung injury scores were calculated by adding the individual scores for each category. Notice that all the identities of the groups were blind to the three investigators.

Table 2
Animal grouping and SB239063 administration.

Groups	Model	HE	WB	Lung edema
Control	II/R + normal saline	8	8	8
Inhibitor	II/R + SB239063	8	8	8

HE, hematoxylin and eosin stain; WB, western blotting; II/R, intestinal ischemia/reperfusion, including reperfusion 16 h, 24 h, 48 h.