Inhibition of Na-K-Cl cotransporter isoform 1 reduces lung injury induced by ischemia–reperfusion

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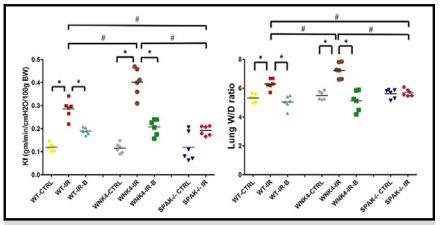
ABSTRACT

Objectives: Ischemia–reperfusion acute lung injury is characterized by increased vascular permeability, lung edema, and neutrophil sequestration. Ischemia–reperfusion acute lung injury occurs in lung transplantation and other major surgical procedures. Effective regulation of alveolar fluid balance is critical for pulmonary edema. Sodium-potassium-chloride co-transporter regulates alveolar fluid and is associated with inflammation. We hypothesized that sodium-potassium-chloride co-transporter is important in ischemia–reperfusion acute lung injury. Bumetanide, a sodium-potassium-chloride co-transporter inhibitor, is used to treat pulmonary edema clinically. We studied the effect of bumetanide in ischemia–reperfusion acute lung injury.

Methods: Isolated perfusion of mouse lungs in situ was performed. The main pulmonary artery and left atrium were catheterized for lung perfusion and effluent collection for recirculation, respectively, with perfusate consisting of 1 mL blood and 9 mL physiologic solution. Ischemia–reperfusion was induced by 120 minutes of ischemia (no ventilation or perfusion) and reperfused for 60 minutes. Wild-type, SPAK knockout ($SPAK^{-/-}$), and WNK4 knockin ($WNK4^{D561A/+}$) mice were divided into control, ischemia–reperfusion, and ischemia–reperfusion + bumetanide groups (n = 6 per group). Bumetanide was administered via perfusate during reperfusion. Measurements were taken of lung wet/dry weight, microvascular permeability, histopathology, cytokine concentrations, and activity of the nuclear factor-κB pathway.

Results: In wild-type mice, ischemia–reperfusion caused lung edema (wet/dry weight 6.30 ± 0.36) and hyperpermeability (microvascular permeability, 0.29 ± 0.04), neutrophil sequestration (255.0 ± 55.8 cells/high-power field), increased proinflammatory cytokines, and nuclear factor-κB activation (1.33 ± 0.13). Acute lung injury was more severe in WNK4 mice with more lung edema, permeability, neutrophil sequestration, and nuclear factor-κB activation. Severity of acute lung injury was attenuated in $SPAK^{-/-}$mice. Bumetanide decreased pulmonary edema (wild-type: wet/dry weight 5.05 ± 0.44, WNK4: wet/dry weight 5.13 ± 0.70), neutrophil sequestration (wild-type: 151.7 ± 27.8 cells/high-power field, WNK4: 135.3 ± 19.1 cells/high-power field), permeability (wild-type: 0.19 ± 0.01, WNK4: 0.21 ± 0.03), cytokines, and nuclear factor-κB activation after ischemia–reperfusion.



Higher NKCC1 leads to severe lung injury with higher edema, permeability, and inflammation.

Central Message

Higher NKCC1 leads to more severe ALI with greater lung edema, permeability, and neutrophil sequestration. Manipulations that decrease NKCC1 can attenuate the severity of lung injury.

Perspective

NKCC1 regulates ion transport and fluid balance in the lungs. NKCC1 may play a critical role in ALI. However, the study on the role of NKCC1 in IR-induced ALI is lacking. Our study found that NKCC1 plays an important role in IR-induced ALI. Genetic and pharmacologic manipulation to decrease NKCC1 can attenuate ALI by suppressing the NF-κB pathway.

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Abbreviations and Acronyms

ALI = acute lung injury
BALF = bronchoalveolar lavage fluid
BW = body weight
IκB = inhibitor of NF-κB
IKK = IκB kinase
IR = ischemia–reperfusion
Kf = microvascular permeability
KNCC = sodium-potassium-chloride co-transporter
LW = lung weight
MAPK = mitogen activated protein kinase
MIP-2 = macrophage inflammatory protein-2
NF-κB = nuclear factor-kappaB
OSR1 = oxidative stress responsive kinase-1
PA = pulmonary artery
PAP = pulmonary artery pressure
PBS = phosphate-buffered saline
PVP = pulmonary venous pressure
SPAK = STE20/SPS1-related proline/alanine rich kinase
TNF-α = tumor necrosis factor-α
W/D = wet/dry weight ratio (W/D)
WT = wild-type

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Conclusions: Functional reduction of sodium-potassium-chloride co-transporter by genetic or pharmacologic treatment to inhibit sodium-potassium-chloride co-transporter resulted in lower severity of acute lung injury induced by ischemia–reperfusion. Sodium-potassium-chloride co-transporter may present a promising target for therapeutic interventions in a clinical setting. (J Thorac Cardiovasc Surg 2017;153:206-15)

Acute lung injury (ALI) induced by ischemia–reperfusion (IR) occurs when the lungs are exposed to periods of IR.[1] ALI is characterized by increased pulmonary vascular permeability, lung edema, neutrophil sequestration, and hypoxemia.[1] IR-induced ALI occurs in many clinical situations, including lung transplantation, cardiopulmonary bypass, and other major surgical procedures.[2,3] Lung transplantation provides a curative therapy for patients with end-stage lung diseases. However, the shortage of donor organs remains a major limiting factor in the widespread application of lung transplantation.[4] Despite advances in organ preservation and perioperative care, IR-induced ALI remains a significant cause of mortality and morbidity after lung transplantation.[4] Therefore, there is an increasing effort to study IR-induced ALI to improve outcomes in lung transplantation.[5,6]

Alveolar fluid regulation is considered to have a critical role in the development of pulmonary edema in lung injury. Ion channels in the lung epithelium regulate water influx and efflux and are likely to be important in IR-induced ALI. However, previous studies on the role of ion channels in IR-induced ALI have been inconclusive, and the role of ion channels in ALI is still not fully understood. To gain a better understanding of the role of fluid regulation in lung injury, studies that address the underlying mechanisms and the involvement of ion channels will be important. This may lead to new therapeutic options for the management of ALI.

The sodium-potassium-chloride co-transporter (NKCC) regulates intracellular volume by coupling the transport of sodium (Na^+), chloride (Cl^-), and potassium (K^+).[7] Two isoforms of the NKCC have been identified: NKCC1 and NKCC2. Only NKCC1 is expressed in alveolar epithelial cells, whereas NKCC2 expression is restricted to the kidney.[7] NKCC1 is located basolaterally in the lung epithelium that mediates a net influx of ions into cells.[8] Therefore, the chemical gradient that creates the driving force for water transport is mediated primarily by NKCC1.[8]

The activity of epithelial NKCC1 is regulated by a phosphorylation signaling cascade consisting of with-no-lysine kinase (WNK), oxidative stress responsive kinase-1 (OSR1), and STE20/SPS1-related proline/alanine rich kinase (SPAK).[9] SPAK and OSR1 are downstream substrates of WNK1 and WNK4 kinases and upstream regulators of NKCC1.[9] Activation of WNK4 has been shown to activate the OSR1/SPAK phosphorylation cascade.[9] Phosphorylated OSR1 and SPAK are known to phosphorylate NKCC1.[9] Although it is well documented that the WNK-SPAK pathway plays a crucial role in systemic salt and water homoeostasis,[9] its role in regulating pulmonary fluid clearance is largely unknown. Nuclear factor-kappaB (NF-κB) and proinflammatory cytokines, including interleukin-6, macrophage chemoattractant protein-1, and tumor necrosis factor-α (TNF-α), play important roles in lung injury.[10] A study exploring the central role for NF-κB in the induction of lung injury revealed that NF-κB is a critical regulator of the inflammatory response in lung injury, with inhibition of NF-κB activation, reduction of IR injury, and improvement of pulmonary graft function.[11] NF-κB also plays a critical role in SPAK regulation.[10] During IR, the NF-κB pathway is activated and regulates not only proinflammatory cytokines but also SPAK and NKCC1 activity.[10] Thus, we hypothesized that the pathogenic mechanisms underlying ALI

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