Fingolimod attenuates renal ischemia/reperfusion-induced acute lung injury by inhibiting inflammation and apoptosis and modulating S1P metabolism

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Abstract

Objective: This study examined whether the immunomodulator fingolimod (FTY720) could alleviate renal ischemia/reperfusion (I/R)-induced lung injury and explored the potential mechanisms.

Methods: Renal I/R was established in a rat model, and FTY720 (0.5, 1, or 2 mg/kg) was injected intraperitoneally after 15 minutes of ischemia. Pro-inflammatory cytokine levels, oxidative stress, apoptosis, and the mRNA expression of the sphingosine-1-phosphate (S1P)-related signaling pathway genes sphingosine kinase-1 (SphK1) and sphingosine kinase-2 were analyzed in lung tissue.

Results: Increased pro-inflammatory cytokine levels; decreased total superoxide dismutase, catalase, and glutathione peroxidase levels; increased apoptosis; and increased S1P lyase and SphK1 expression were observed following renal I/R. FTY720 reversed renal I/R-induced changes and effectively attenuated lung injury.

Conclusion: FTY720 protected against acute lung injury in rats subjected to renal I/R by decreasing pulmonary inflammation and apoptosis, increasing oxidative stress, and modulating S1P metabolism.

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Introduction

Renal ischemia/reperfusion (I/R) injury is most commonly associated with acute renal impairment, kidney transplantation, and/or extracorporeal shock wave lithotripsy.[1,2] Additionally, patients often present with distant organ damage following renal I/R.[3] This effect is partly mediated by the diminished exclusion of inflammatory cytokine release. Acute lung injury is the one of the major causes of death in patients with renal I/R injury.[4,5]

Multiple pathophysiological processes are involved in renal I/R-induced lung injury. Elevated levels of inflammatory mediators cause disturbances in microcirculation, increased capillary permeability, destruction of cell membranes and lysosomes, cell edema, and tissue damage leading to lung injury.[6] To date, there are no preventative therapies targeting lung injury caused by renal I/R. Furthermore, treatment strategies that maintain oxygenation capacity and pulmonary mechanics following renal I/R are limited.

Fingolimod (FTY720), a sphingosine-1-phosphate (S1P) receptor agonist, is a promising immunomodulator that could interfere with the pathogenesis of I/R-induced injury.[7–9] FTY720 is a phosphorylation target for sphingosine kinase-2 (SphK2) that binds to S1P receptors 1 to 5.[10] The S1P signaling pathway is involved in various biological functions, including cell proliferation and survival, extracellular matrix assembly, endothelial cell barrier integrity, and immune cell trafficking.[11–13]

In animal models of lung injury induced by hindlimb I/R; lipopolysaccharide, paraquat, or bleomycin exposure; or lung transplantation, FTY720 treatment significantly decreased lung leak, inflammation, and endothelial cell apoptosis.[7,14–16] However, whether FTY720 has similar beneficial effects on renal I/R-induced lung injury remains unknown.

In the current study, we establish a rat model of lung injury induced by renal I/R and explored the impact of FTY720 treatment on key pathophysiological processes including inflammatory responses, oxidative stress, apoptosis, and S1P-related signaling.

Materials and methods

Experimental animals

Adult Sprague–Dawley rats (200–300 g) were obtained from the laboratory animal center of North Sichuan Medical College. Rats were housed individually in cages. All rats were housed under a 12-hour/12-hour light/dark cycle at 20 to 24°C with free access to water and food. The protocol of the study was approved by the Ethics Review Board for Animal Studies of North Sichuan Medical College (NSMC2018 45). The study complied with the guidelines for laboratory animal experiment of the National Institutes of Health.

Experimental design and renal I/R model

Twenty-five rats were assigned randomly into five groups (n = 5 per group) as

follows: sham-operated group (Sham group), renal I/R group (RIR group), renal I/R + FTY720 (0.5 mg/kg) group (FTY0.5 group), renal I/R + FTY720 (1 mg/kg) group (FTY1 group), and renal I/R + FTY720 (2 mg/kg) group (FTY2 group). The rat model of renal I/R was established successfully according to a previously described protocol.[17] Briefly, after an injection of sodium pentobarbital (50 mg/kg, i.p.), a midline laparotomy was performed, and the right kidney was removed. Then, a non-traumatic microvascular clamp was applied to occlude the left renal hilum and induce renal ischemia. Reperfusion was allowed after the clip was held for 45 minutes. The same laparotomy procedure was performed in the Sham group without removing the right kidney and clipping the left renal pedicle. Rats in the FTY0.5, FTY1, and FTY2 groups received peritoneal injections of 0.5, 1, and 2 mg/kg FTY720 (Cayman Chemical, Ann Arbor, MI, USA), respectively, 30 minutes before reperfusion, whereas animals in the Sham and RIR groups received normal saline. The wound was perfused with bupivacaine (0.25%) and closed with sterile suture. Rectal temperature was maintained at 36 to 37°C during surgery. All rats were housed individually after recovering from anesthesia. Finally, rats were euthanized with pentobarbital sodium (60 mg/kg i.p.) 6 hours post-reperfusion. Left renal tissue, bronchoalveolar lavage fluid (BALF), and lung tissue were harvested for examination.

Histopathologic examination of kidney

Renal samples were fixed in 10% neutral-buffered formalin for 24 hours, embedded in paraffin, and sectioned into 5-μm-thick slices. Subsequently, hematoxylin–eosin (H&E) staining was performed using the standard protocol, and the pathological alterations were assessed by a pathologist using a light microscope (Olympus, Tokyo, Japan) in a blinded manner.[4] Renal tissue was histopathologically examined for the degree of Bowman's space enlargement, tubular cell necrosis, cell exfoliation in the lumen, vascular congestion, and intratubular cast formation. The level of each manifestation was graded according to the extent of change as follows: 0, no change; 1, <20% change; 2, 20% to 40% change; 3, 41% to 60% change; 4, 61% to 80% change; and 5, >80% change. The sum of all numerical scores in each group was taken as the total histopathological score.

Analysis of renal function

Serum cystatin C (Cys C), serum creatinine (Scr), and blood urea nitrogen (BUN) levels were measured using an autoanalyzer (Siemens, Munich, Germany).

Measurement of inflammatory mediator levels in BALF and the lungs

BALF was collected by perfusing the lungs with 4 mL of PBS through a tracheal cannula placed in each rat under anesthesia. The collection procedure was repeated three times. Briefly, the chest was opened after sacrifice. The trachea was isolated via blunt dissection after median sternotomy. A small-caliber tube was inserted into the airway. PBS was slowly infused bilaterally into the lungs. Then, BALF was withdrawn into the tubes. The percent fluid recovery exceeded 80%. BALF samples were centrifuged ($1500 \times g$) for 10 min at 4°C. The concentrations of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 in BALF and lung tissue were measured using commercial enzyme-linked immunosorbent assay kits (Shanghai Zcibio Biotechnology Ltd., Shanghai, China).

Measurement of oxidative stress in the lungs

Lung tissue was harvested, homogenized, and centrifuged. Supernatant was collected, and the levels of total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-PX), catalase (CAT), and malondialdehyde (MDA) were determined using corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer s instructions.

Measurement of neutrophil infiltration in the lungs

A formalin-fixed left lower lung lobe tissue sample was prepared from each animal. Samples were dehydrated and embedded in Paraplast (Leica Microsystems GmbH, Wetzlar, Germany). The tissues were cut into 4-μm-thick sections and stained with H&E. Lung neutrophil infiltration was examined by a pathologist who was blinded to the group allocation under a light microscope.

Assessment of cell apoptosis

Lung tissues were fixed with 4% paraformaldehyde, embedded in paraffin, and sectioned at 5 μm. After deparaffinization and rehydration, the sections were treated with 10 μg/mL protease K at 37°C for 25 minutes. Fresh terminal deoxynucleotidyl transferase-mediated dUDP nick-end labeling (TUNEL) reaction mixture was added onto the samples, which were then incubated at 37°C for 60 minutes. Apoptosis was measured using a TUNEL assay kit (Roche, Basel, Switzerland).

Measurement of mRNA expression of S1P-related signaling pathway genes

The right lower lung lobe tissue samples of each animal were homogenized. Total RNA was extracted using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. For real-time PCR, the total RNA (1 μg) from each sample was resuspended in 25 μL of reaction buffer, and cDNA was reverse-transcribed using a synthesis kit (Takara Biotechnology Co. Ltd., Dalian, China). The housekeeping gene β-actin was used for data normalization. Relative mRNA expression was measured using the $2^{-\Delta\Delta CT}$ method. The primers used for real-time PCR were as follows: S1P lyase (S1PL) forward primer, 5′-ATT GGC AAG GTG GCA TCT ACG CAT AT-3′; S1PL reverse primer, 5′-CCA GTT CTG ACT TGA GGA AGC GAG CA-3′; SphK1 forward primer, 5′-TCG TTC TGG TGC TGG TGC TGC TTC A-3′; sphingosine kinase-1 (SphK1) reverse primer, 5′-GGA AGG CAA CCA CTG GCA CAT GAA CC-3′; SphK2 forward primer, 5′-GGG TTG AGC CTG AGT GAG TGG GAA G-3′; SphK2 reverse primer, 5′-CGG CAG AGA AGA AGC GAG CAG TTG A-3′; β-actin forward primer, 5′-GAA GAT CAA GAT CAT TGC TCC T-3′; and β-actin reverse primer, 5′-TAC TCC TGC TTG CTG ATC CA-3′.

Statistical analysis

All statistical analyses were performed using SPSS 23.0 (IBM, Armonk, NY, USA). Continuous variables are presented as the mean ± SD. Statistical significance was evaluated using analysis of variance followed by Tukey's multiple comparison test. $P < 0.05$ was considered significant.

Results

Effects of FTY720 on renal injury

Renal injury was assessed *via* histopathologic examination and assessments of renal function. Rats subjected to renal I/R

exhibited significant structural damage compared with rats in the Sham group (Figure 1d and e) and Cys C (0.50 ± 0.02 mg/L vs. 0.26 ± 0.03 mg/L), Scr (60.75 ± 6.08 µmol/L vs. 39.33 ± 10.02 µmol/L), and BUN levels (10.90 ± 1.96 mmol/L vs. 6.94 ± 0.85 mmol/L) were significantly higher in the RIR group than in the Sham group (all $P < 0.05$; Figure 1a–c). However, treatment with 0.5 mg/kg FTY720 significantly decreased Cys C and Scr levels (0.27 ± 0.06 mg/L and 42.00 ± 4.47 µmol/L,

respectively; both $P < 0.05$) and attenuated pathological injuries (Figure 1a, b, and f).

Effects of FTY720 on pro-inflammatory cytokine production in BALF and lung tissue

Significantly higher IL-6 (27.66 ± 1.75 pg/mL vs. 14.17 ± 0.82 pg/mL), IL-1β (12.75 ± 0.52 pg/mL vs. 10.85 ± 0.91 pg/mL), and TNF-α levels (110.93 ± 5.48 pg/mL vs. 93.19 ± 3.65 pg/mL) were observed
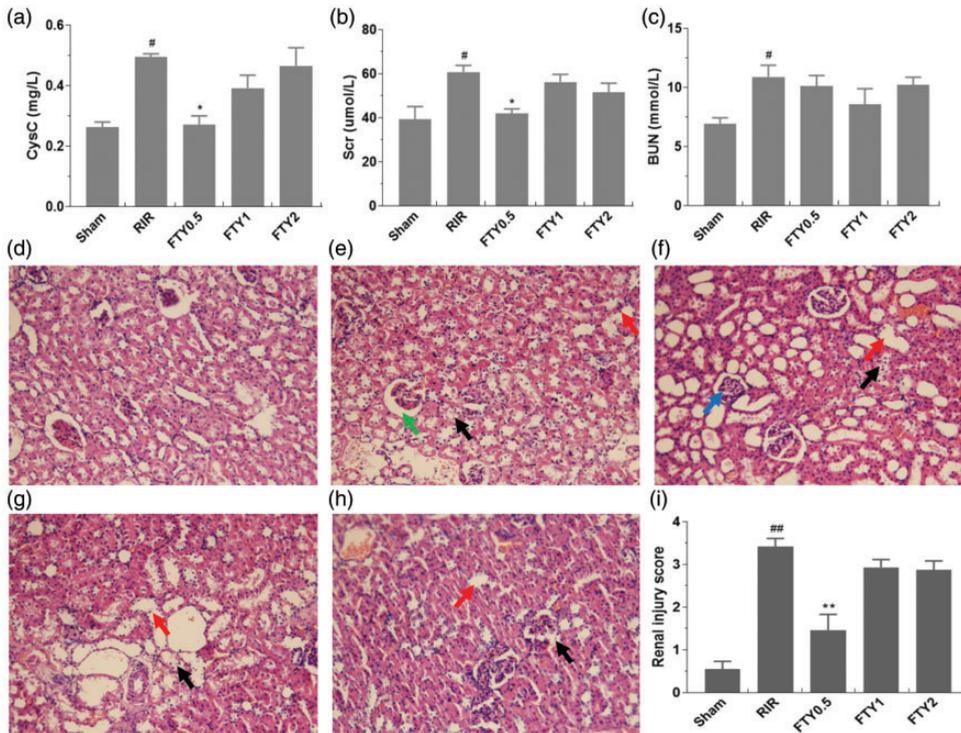
Figure 1. Serum levels of (a) Cys C), (b) Scr, and (c) BUN in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups. Representative microscopic findings of the left kidney (d–h) after renal I/R in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups, respectively (hematoxylin and eosin, ×200 magnification), including the enlargement of Bowman's space (green arrow), necrosis and exfoliation of proximal tubular cells in the lumen (black arrow), dilated renal tubules and flattened cells (red arrow), and significantly reduced red blood cell counts in glomerular capillaries (blue arrow). (i) Histological injury scores for left kidney tissue. Data are presented as the mean ± SD. #$P < 0.05$ vs. the Sham group; *$P < 0.05$ vs. the RIR group; ##$P < 0.01$ vs. the Sham group; **$P < 0.01$ vs. the RIR group.
Cys C, cystatin C; Scr, creatinine; BUN, blood urea nitrogen; Sham, sham-operated group; RIR, renal I/R; FTY0.5, renal I/R + 0.5 mg/kg FTY720 group; FTY1, renal I/R + 1 mg/kg FTY720 group; FTY2, renal I/R + 2 mg/kg FTY720 group; I/R, ischemia/reperfusion; SD, standard deviation.

in BALF from the RIR group than in BALF from the Sham group (all $P < 0.01$; Figure 2a–c). However, IL-6, IL-1β, and TNF-α levels were significantly decreased in the FTY0.5 group (16.77 ± 1.07, 11.56 ± 0.38, and 94.65 ± 1.82 pg/mL, respectively, all $P < 0.05$; Figure 2a–c). Similarly, the RIR group displayed significantly higher IL-6 (39.22 ± 2.98 pg/mL vs. 21.51 ± 1.22 pg/mL) IL-1β (13.45 ± 0.55 pg/mL vs. 11.71 ± 0.22 pg/mL), and TNF-α levels (103.43 ± 6.23 pg/mL vs. 89.30 ± 5.65 pg/mL) in lung tissue than the Sham group (all $P < 0.01$; Figure 3a–c). By contrast, FTY720 at 0.5 and 1 mg/kg significantly suppressed the elevated levels of IL-

6 (31.02 ± 1.94 and 29.64 ± 1.36 pg/mL, respectively), IL-1β (11.95 ± 0.23 and 12.07 ± 0.15 pg/mL, respectively), and TNF-α (92.55 ± 3.38 and 92.01 ± 3.94 pg/mL, respectively; all $P < 0.05$; Figure 3a–c).

Effects of FTY720 on oxidative stress in the lungs

The RIR group exhibited significantly lower T-SOD (46.85 ± 2.11 U/mg protein vs. 52.95 ± 0.71 U/mg protein), CAT (6.77 ± 0.33 U/mg protein vs. 8.85 ± 0.41 U/mg protein), and GSH-PX levels (81.29 ± 2.99 U/mg protein vs. 119.47 ± 7.40 U/mg protein) and notably higher MDA levels
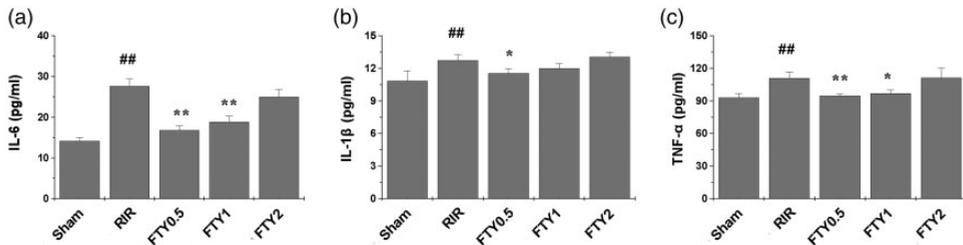
Figure 2. Bronchoalveolar lavage fluid levels of IL-6 (a), IL-1β (b), and TNF-α (c) in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups. Data are presented as means ± SD. ##$P < 0.01$ vs. the Sham group; **$P < 0.01$ vs. the RIR group; *$P < 0.05$ vs. the RIR.
IL, interleukin; TNF-α, tumor necrosis factor-α; Sham, sham-operated group; RIR, renal I/R group; FTY0.5, renal I/R + 0.5 mg/kg FTY720 group; FTY1, renal I/R + 1 mg/kg FTY720 group; FTY2, renal I/R + 2 mg/kg FTY720 group; SD, standard deviation.
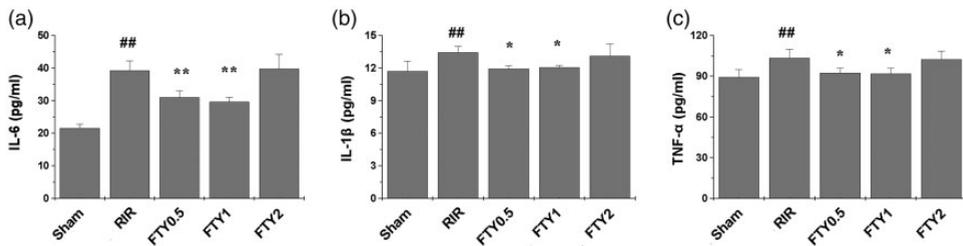
Figure 3. Lung levels of IL-6 (a), IL-1β (b), and TNF-α (c) in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups. Data are presented as the mean ± SD. ##$P < 0.01$ *vs.* the Sham group; **$P < 0.01$ vs. the RIR group; *$P < 0.05$ vs. the RIR group.
IL, interleukin; TNF-α, tumor necrosis factor-α; Sham, sham-operated group; RIR, renal I/R group; FTY0.5, renal I/R + 0.5 mg/kg FTY720 group; FTY1, renal I/R + 1 mg/kg FTY720 group; FTY2, renal I/R + 2 mg/kg FTY720 group; SD, standard deviation.

(0.92 ± 0.04 U/mg protein vs. 0.75 ± 0.09 U/mg protein) in lung tissue than the Sham group (all $P < 0.01$; Figure 4a–d). These changes were reversed in the FTY0.5 group (T-SOD, 49.39 ± 1.83 U/mg protein; CAT, 7.73 ± 0.72 U/mg protein; GSH-PX, 105.68 ± 7.18 U/mg protein; MDA, 0.84 ± 0.04 U/mg protein; all $P < 0.05$; Figure 4a–d).

Effects of FTY720 on apoptosis in the lungs

The extent of apoptosis was significantly increased in the RIR group compared with the findings in the Sham group (apoptotic area: 10.89 ± 0.46 vs. 5.83 ± 0.66; all $P < 0.01$; Figure 5a, b, and f). However, this increase in apoptosis was reversed in all rats receiving FTY720 (apoptotic area: 6.06 ± 0.93, 4.45 ± 0.58, and 4.56 ± 1.15 in the FTY0.5, FTY1, and FTY2 groups, respectively, all $P < 0.01$; Figure 5c–f).

Effects of FTY720 on the mRNA expression of S1P-related signaling pathway genes

The mRNA expression of S1PL (2.94 ± 0.83 vs. 1.01 ± 0.13), SphK1 (4.01 ± 0.73 vs. 1.02 ± 0.21), and SphK2 (2.64 ± 0.84 vs. 1.11 ± 0.43) was significantly higher in the RIR group than in the Sham group (all $P < 0.05$; Figure 6a–c). However, in the FTY0.5 and FTY1 groups, S1PL (1.36 ± 0.27 and 1.58 ± 0.52, respectively), SphK1 (1.50 ± 0.29 and 1.88 ± 0.31, respectively) and SphK2 mRNA expression (1.24 ± 0.30 and 1.43 ± 0.44, respectively) was significantly downregulated versus that in the Sham group (all $P < 0.05$; Figure 6a–c).
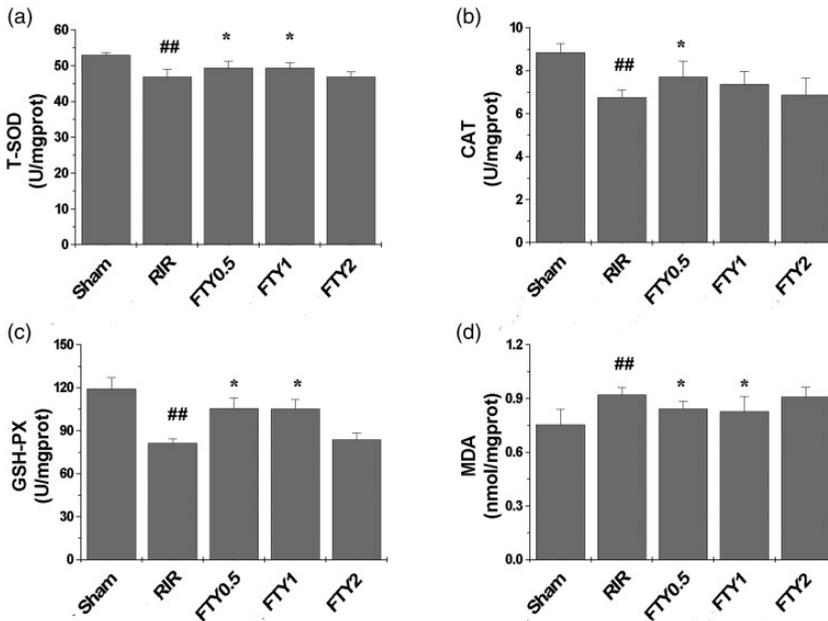
Figure 4. Lung levels of T-SOD (a), CAT (b), GSH-PX (c), and MDA (d) in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups. Data are presented as the mean ± SD. $^{##}P < 0.01$ vs. the Sham group; $^{*}P < 0.05$ vs. the RIR group.
T-SOD, total superoxide dismutase; CAT, catalase; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; Sham, sham-operated group; RIR, renal I/R group; FTY0.5, renal I/R + 0.5 mg/kg FTY720 group; FTY1, renal I/R + 1 mg/kg FTY720 group; FTY2, renal I/R + 2 mg/kg FTY720 group; SD, standard deviation.
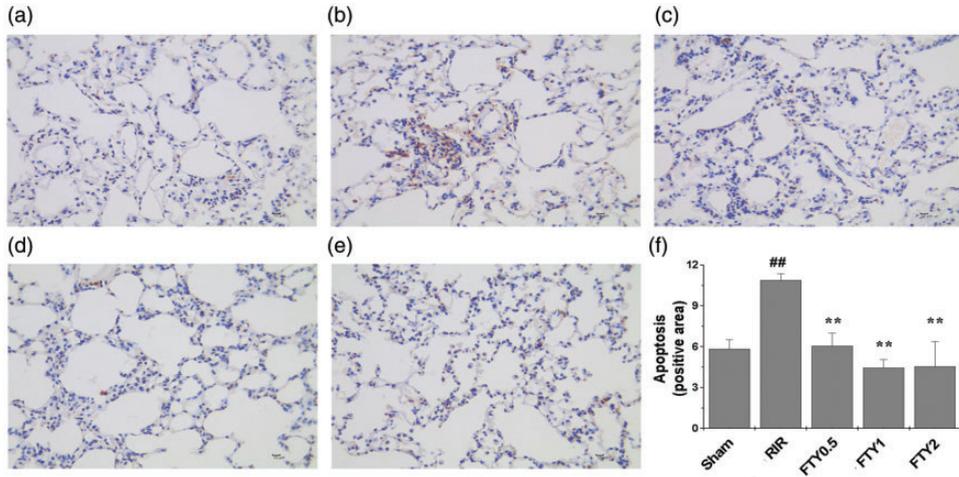
Figure 5. Apoptosis of lung cells in the (a) Sham, (b) RIR, (c) FTY0.5, (d) FTY1, and (e) FTY2 groups ($\times 400$ magnification). (f) The positive apoptotic area. Data are presented as the mean $\pm$ SD. $^{\#\#}P < 0.01$ vs. the Sham group; $^{**}P < 0.01$ vs. the RIR group.
Sham, sham-operated group; RIR, renal I/R group; FTY0.5, renal I/R $+ 0.5$ mg/kg FTY720 group; FTY1, renal I/R $+ 1$ mg/kg FTY720 group; FTY2, renal I/R $+ 2$ mg/kg FTY720 group; SD, standard deviation.
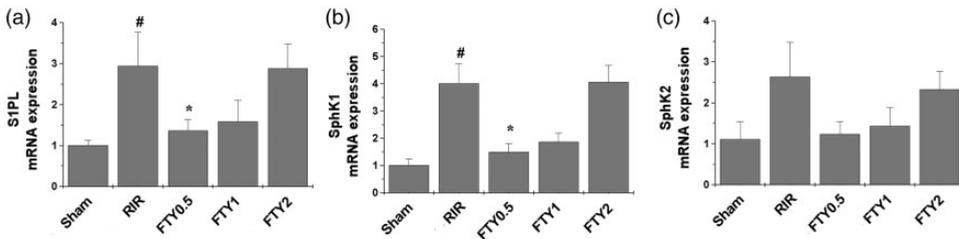
Figure 6. mRNA expression of S1PL (a), SphK1 (b), and SphK2 (c) in lung tissue in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups. Data are presented as the mean $\pm$ SD. $^{\#}P < 0.05$ vs. the Sham group; $^{*}P < 0.05$ vs. the RIR group.
S1PL, S1P lyase; SphK1, sphingosine kinase-1; SphK2, sphingosine kinase-2; Sham, sham-operated group; RIR, renal I/R group; FTY0.5, renal I/R $+ 0.5$ mg/kg FTY720 group; FTY1, renal I/R $+ 1$ mg/kg FTY720 group; FTY2, renal I/R $+ 2$ mg/kg FTY720 group; SD, standard deviation.

Effects of FTY720 on neutrophil infiltration in the lungs

Neutrophil infiltration in the lungs was significantly increased in the RIR group compared with that in the Sham group (9.58 ± 1.36 vs. 6.67 ± 1.03; $P < 0.01$; Figure 7a, 7b, and 7f). This increase was attenuated in the FTY0.5, FTY1, and FTY2 groups (5.92 ± 0.80, 6.25 ± 1.04, and 6.93 ± 0.83, respectively; $P < 0.01$; Figure 7c–f), although no significant differences in neutrophil infiltration were identified among these three groups.

Discussion

The current study evaluated the effect of FTY720 on renal I/R-induced lung injury in a rat model. Our findings confirmed
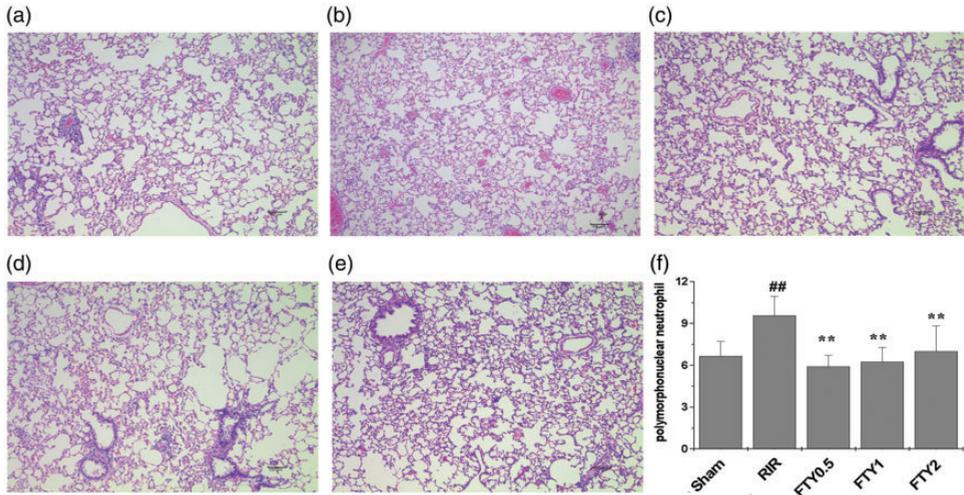
Figure 7. Neutrophil infiltration in lungs in the Sham, RIR, FTY0.5, FTY1, and FTY2 groups (×200 magnification). Data are presented as the mean ± SD. ##$P < 0.01$ vs. the Sham group; **$P < 0.01$ vs. the RIR group.

Sham, sham-operated group; RIR, renal I/R group; FTY0.5, renal I/R + 0.5 mg/kg FTY720 group; FTY1, renal I/R + 1 mg/kg FTY720 group; FTY2, renal I/R + 2 mg/kg FTY720 group; SD, standard deviation.

that the activation of multiple responses, including inflammation, oxidative stress, and apoptosis, triggered a sequence of events leading to acute lung injury. FTY720 effectively attenuated lung injury caused by renal I/R by inhibiting inflammatory cytokine production and neutrophil infiltration, suppressing apoptosis and oxidative stress, and modulating S1P metabolism.

Inflammatory cytokines including TNF-α, IL-6, and IL-1β play critical roles in the recruitment and activation of inflammatory cells in response to acute lung injury.[18] TNF-α plays a key role in mitochondrial toxicity, apoptosis, neutrophil activation, and necrotic cell death in I/R-mediated lung injury.[19] IL-6 is regarded as an early biomarker of tissue damage reflecting the accumulation of neutrophils in the lungs. IL-6 and TNF-α further stimulate inflammatory cytokine release, resulting in an inflammatory cascade. Prior research revealed that cytokines and inflammatory

pathways are responsible for the pathogenesis of multiple-organ failure following abdominal aortic aneurysm repair.[20] Lower-extremity I/R-induced lung injury stimulated IL-1β, which is also an important mediator in I/R. In our model of renal I/R-induced lung injury, the levels of these inflammatory cytokines were significantly elevated in BALF and lung tissue, supporting the histological findings of alveolar capillary membrane disruption, interstitial edema, and neutrophil infiltration. The present study found that the S1P receptor agonist FTY720 significantly curbed the production of TNF-α, IL-6, and IL-1β in BALF and lung tissues, which was consistent with previous studies on FTY720 in the treatment of lung injury.[15] FTY720 may prevent cytokine-mediated acute inflammatory responses in renal I/R-induced lung injury.

The lungs are vulnerable to oxidative stress because of their unique structure and function.[21] A previous study reported

that MDA levels in BALF and lung tissue were significantly elevated after aortic I/R injury.[22] Our results suggested that accumulated MDA contributes to renal I/R-induced lung injury, which is consistent with previous findings.[23] In the present study, decreased MDA levels and increased T-SOD, CAT, and GSH-PX levels were observed after FTY720 treatment, indicating that FTY720 effectively elicits a protective response to oxidative stress.

Apoptosis following I/R is equally important in the development of lung injury.[24] Previous studies on I/R injury during lung transplantation found that the mode of pulmonary epithelial cell death upon reperfusion after a moderate duration (6 or 12 hours) of cold ischemic preservation was dominated by apoptosis.[25] Conversely, apoptosis was clearly observed in lung tissue after 45 minutes of ischemia followed by 6 hours of reperfusion. Previous findings indicated that apoptosis in the alveolar epithelium is evident only after reperfusion, and the degree of apoptosis increases with longer periods of reperfusion, in line with the findings of the current study.[26] The binding of S1P to its receptors may inhibit apoptosis.[27] Therefore, it was not surprising that FTY720 reduced pulmonary cell apoptosis.

It is noteworthy that FTY720 may have therapeutic limitations despite its beneficial effects because it induces the ubiquitination and proteasomal degradation of S1P$_1$, leading to increased endothelial permeability after prolonged exposure.[10] This may explain why higher concentrations failed to enhance the protective effects of FTY720 and potentially exerted detrimental effects on vascular permeability. Indeed, several recent animal studies observed detrimental effects on vascular permeability when FTY720 was applied at higher concentrations and for longer periods, leading

to tissue edema and worsening bleomycin-induced lung injury and ventilator-induced lung injury.[14,28,29] This experiment indicated that renal I/R caused kidney injury, and this damage was attenuated by FTY720 (0.5 mg/kg). However, higher concentrations of FTY720 (1 and 2 mg/kg) did not aggravate renal damage.

Our study had several limitations. First, FTY720 is one of several S1P receptor agonists, and the effects of other S1P receptor agonists on renal I/R-induced lung injury were not evaluated. Second, FTY720 is clinically administered in an oral form, and the bioavailability of the active agent is affected by first-pass metabolism; therefore, the appropriate FTY720 dosage to elicit protective effects needs to be further evaluated. In addition, in this experimental study, we performed FTY720 pretreatment to ensure a sufficient dose and effect. Posttreatment with the treatment might better mimic the clinical setting. Finally, the long-term effects of FTY720 on lung injury caused by renal I/R remain unknown because of the short follow-up period of this study. Overall, the present study suggests that FTY720 at a low concentration (0.5 mg/kg) alleviates renal I/R-induced lung injury in rats by attenuating inflammation, oxidative stress, and apoptosis and modulating S1P metabolism. These observations indicate that FTY720 may be a promising treatment for acute lung injury after renal I/R.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Hou J, Rao M, Zheng W, et al. Advances on cell autophagy and its potential regulatory factors in renal ischemia-reperfusion injury. *DNA Cell Biol* 2019; 38: 895–904.
2. Dzięgała M, Krajewski W, Kołodziej A, et al. Evaluation and physiopathology of minor transient shock wave lithotripsy - induced renal injury based on urinary biomarkers levels. *Cent European J Urol* 2018; 71: 214–220.
3. Lee SA, Martina C, Bush EL, et al. Distant organ dysfunction in acute kidney injury: a review. *Am J Kidney Dis* 2018; 72: 846–856.
4. Karimi Z, Ketabchi F, Alebrahimdehkordi N, et al. Renal ischemia/reperfusion against nephrectomy for induction of acute lung injury in rats. *Ren Fail* 2016; 38: 1503–1515.
5. Du M, Yang L, Gu J, et al. Inhibition of peptidyl arginine deiminase-4 prevents renal ischemia-reperfusion-induced remote lung injury. *Mediators Inflamm* 2020; 2020: 1724206.
6. Collange O, Fabienne T, Nathalie R, et al. Pulmonary apoptosis after supraceliac aorta clamping in a rat model. *J Surg Res* 2005; 129: 190–195.
7. Wang LR, Chen FF, Pan YF, et al. Effects of FTY720 on lung injury induced by hindlimb ischemia reperfusion in rats. *Mediators Inflamm* 2017; 2017: 5301312.
8. Foster AD, Vicente D, Clark N, et al. FTY720 effects on inflammation and liver damage in a rat model of renal ischemia-reperfusion injury. *Mediators Inflamm* 2019; 2019: 3496836.
9. Bonitz JA, Son JY, Chandler B, et al. A sphingosine-1 phosphate agonist (FTY720) limits trauma/hemorrhagic shock-induced multiple organ dysfunction syndrome. *Shock* 2014; 42: 448–455.
10. Pérez-Jeldres T, Alvarez-Lobos M and Rivera-Nieves J. Targeting sphingosine-1-phosphate signaling in immune-mediated diseases: beyond multiple sclerosis. *Drugs* 2021; 81: 985–1002.
11. Huwiler A and Zangemeister-Wittke U. The sphingosine 1-phosphate receptor modulator fingolimod as a therapeutic agent: Recent findings and new perspectives. *Pharmacol Ther* 2018; 185: 34–49.
12. Luo YT, Liang YF, He H, et al. The immunosuppressant fingolimod ameliorates experimental autoimmune myasthenia gravis by regulating T-cell balance and cytokine secretion. *Am J Transl Res* 2020; 12: 2600–2613.
13. Obinata H and Hla T. Sphingosine 1-phosphate in coagulation and inflammation. *Semin Immunopathol* 2012; 34: 73–91.
14. Wang L, Sammani S, Moreno-Vinasco L, et al. FTY720 (s)-phosphonate preserves sphingosine 1-phosphate receptor 1 expression and exhibits superior barrier protection to FTY720 in acute lung injury. *Crit Care Med* 2014; 42: e189–e199.
15. Qian J, Ye Y, Lv LX, et al. FTY720 attenuates paraquat-induced lung injury in mice. *Int Immunopharmacol* 2014; 21: 426–431.
16. Zhang ZL, Li WF, Heng ZZ, et al. Combination therapy of human umbilical cord mesenchymal stem cells and FTY720 attenuates acute lung injury induced by lipopolysaccharide in a murine model. *Oncotarget* 2017; 8: 77407–77414.
17. Delbridge MS, Shrestha BM, Raftery AT, et al. FTY720 reduces extracellular matrix expansion associated with ischemia-reperfusion induced injury. *Transplant Proc* 2007; 39: 2992–2996.
18. Ologunde R, Zhao H, Lu K, et al. Organ cross talk and remote organ damage following acute kidney injury. *Int Urol Nephrol* 2014; 46: 2337–2345.
19. Yang Z, Zhang XR, Zhao Q, et al. Knockdown of TNF−α alleviates acute lung injury in rats with intestinal ischemia and reperfusion injury by upregulating IL−10 expression. *Int J Mol Med* 2018; 42: 926–934.
20. Katseni K, Chalkias A, Kotsis T, et al. The effect of perioperative ischemia and reperfusion on multiorgan dysfunction following

abdominal aortic aneurysm repair. *Biomed Res Int* 2015; 2015: 598980.

21. Ambruso SL, Gil HW, Fox B, et al. Lung metabolomics after ischemic acute kidney injury reveals increased oxidative stress, altered energy production, and ATP depletion. *Am J Physiol Lung Cell Mol Physiol* 2021; 321: L50–L64.

22. Dou H, Ergene S, Karakii SO, et al. Tea grape reduces abdominal aortic occlusion-induced lung injury. *Braz J Cardiovasc Surg* 2020; 35: 512–520.

23. Erman H, Guner I, Yaman MO, et al. The effects of fluoxetine on circulating oxidative damage parameters in rats exposed to aortic ischemia-reperfusion. *Eur J Pharmacol* 2015; 749: 56–61.

24. Ng CS, Wan S and Yim AP. Pulmonary ischaemia-reperfusion injury: role of apoptosis. *Eur Respir J* 2005; 25: 356–363.

25. Wang X, O'Brien ME, Yu J, et al. Prolonged cold-ischemia induces necroptotic cell death in ischemia reperfusion injury and contributes to primary graft dysfunction after lung transplantation. *Am J Respir Cell Mol Biol* 2019; 61: 244–256.

26. Calabrese F, Schiavon M, Perissinotto E, et al. Organ care system lung resulted in lower apoptosis and inos expression in donor lungs. *Am J Transplant* 2020; 20: 3639–3648.

27. Mohammed S and Harikumar KB. Sphingosine 1-phosphate: a novel target for lung disorders. *Front Immunol* 2017; 8: 296.

28. Oo ML, Chang SH, Thangada S, et al. Engagement of S1P(1)-degradative mechanisms leads to vascular leak in mice. *J Clin Invest* 2011; 121: 2290–2300.

29. Müller HC, Hocke AC, Hellwig K, et al. The Sphingosine-1 Phosphate receptor agonist FTY720 dose dependently affected endothelial integrity in vitro and aggravated ventilator-induced lung injury in mice. *Pulm Pharmacol Ther* 2011; 24: 377–385.