Pulmonary Circulation

Sevoflurane inhibits cardiac function in pulmonary fibrosis mice through the TLR4 signaling pathway

Yanan Cao[1,*], Zhenghua He[1,*], Maoen Zhu[1], Xiaowei Gao[1], Yue Yang[1], Junjie Zhang[1], Yundan Pan[1], Qulian Guo[1], Yonggang Peng[2] and E. Wang[1]

[1]Department of Anesthesiology, Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]Department of Anesthesiology, Shands Hospital, University of Florida, Gainesville, FL, USA

Abstract

Pulmonary fibrosis is often concomitant with myocardial injury. We studied sevoflurane's effects on cardiac function and the expression of the TLR4/inducible nitric oxide synthase (iNOS) signaling pathway on a pulmonary fibrosis model. C57BL/6J wild-type (WT) and TLR4-deficient ($TLR4^{-/-}$) mice were randomly divided into a control group and a pulmonary fibrosis group. The model of pulmonary fibrosis was induced by treatment with paraquat (PQ; 20 mg/kg). Four weeks after PQ administration, mice were tested for body weight changes, and histopathology and hydroxyproline in lung. Left ventricular function in each group of mice was measured by echocardiogram before and after sevoflurane inhalation. The expression of TLR4 and iNOS protein were analyzed. Pulmonary fibrosis mice were fed lenalidomide (50 mg/kg/day) for three days and cardiac function was assessed before and after sevoflurane inhalation. WT pulmonary fibrosis mice showed pathological damage and excessive deposition of collagen in the lung and heart. Left ventricular function decreased after four weeks of PQ exposure. $TLR4^{-/-}$ mice were resistant to pulmonary fibrosis like pathological damage and the effect of sevoflurane on heart rate and ejection fraction than that of WT mice. TLR4 and iNOS expression in WT pulmonary fibrosis mice increased significantly after sevoflurane inhalation. Lenalidomide treatment alleviated the effect of sevoflurane on heart rate and ejection fraction in WT pulmonary fibrosis mice. Sevoflurane inhibits cardiac function in pulmonary fibrosis mice through the TLR4/iNOS pathway. Lenalidomide attenuated the sevoflurane's effect on the cardiac function of mice with pulmonary fibrosis.

Keywords

sevoflurane, cardiac function, pulmonary fibrosis, TLR4

Date received: 7 March 2018; accepted: 24 August 2018

Pulmonary Circulation 2018; 8(4) 1–7
DOI: 10.1177/2045894018800702

Introduction

Pulmonary fibrosis is an irreversible end stage of lung diseases and carries high disability and mortality.[1] From various studies, the probability of pulmonary fibrosis associated with heart disease is in the range of 3–68%.[2] Patients with idiopathic pulmonary fibrosis may experience acute deterioration of cardiopulmonary function after surgery.[3]

Toll-like receptor 4 (TLR4) has been demonstrated to participate in a variety of physiological process such as the immune response, signal transduction, and cell cycle and enzyme regulation.[4–6] Endogenous ligands such as endotoxin, hyaluronic acid, fibronectin, and tenascin-C can bind to TLR4 to recruit MyD88 and release nuclear factor-κβ (NF-κB) via a series of intracellular reactions.[7] Although TLR4 is associated with cardiac dysfunction in sepsis and myocardial infarction, how it is in the cardiac injury of mice with pulmonary fibrosis remains unclear. Sevoflurane has protective effects on myocardial ischemia reperfusion injury;[8,9] however, it also can inhibit myocardial contractility.[10] Studies have shown that sevoflurane protects

*Equal contributors.

Corresponding author:
E. Wang, Department of Anesthesiology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, China.
Emails: ewang324@csu.edu.cn; ewang324@sina.com

the myocardium from ischemia-reperfusion injury through the TLR4/iNOS signaling pathway.[11–13] Increasing iNOS can reduce the contractility of myocardium,[14] tumor necrosis factor-α (TNF-α) can cause an inflammatory response and promote myocardial injury, and a TNF-α inhibitor has been shown to improve cardiac systolic function in obese mice.[15] However, sevoflurane's effects on the cardiac function of mice with pulmonary fibrosis remain to be investigated.

This study explored the mechanisms of sevoflurane's effects on cardiac function in mice with pulmonary fibrosis. We hypothesize that sevoflurane alters TLR4 and iNOS expression and affects the cardiac function of mice with pulmonary fibrosis through the TLR4/iNOS signaling pathway.

Materials and Methods

Animals and pulmonary fibrosis model

Male wild-type (WT) C57BL/6 J mice were provided by the Laboratory Animal Center of Central South University (Changsha, China). Male TLR4-deficient C57BL/10JNju ($TLR4^{-/-}$) mice were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). Male WT and $TLR4^{-/-}$ mice used in the experiments were aged 8–10 weeks and weighed 22–25 g. Mice were fed and housed under controlled temperature (22–25°C), humidity (50–60%), and a 24-h light–dark cycle with access to chow and water ad libitum. All experimental protocols were approved by the Ethics Committee for Animal Research of Central South University. All experimental methods were in accordance with guidelines for treating lab animals.

WT and $TLR4^{-/-}$ mice were intraperitoneally injected with paraquat (PQ; 20 mg/kg, Sigma, St. Louis, MO, USA) to induce a pulmonary fibrosis model. The control mice in each group were intraperitoneally injected with normal saline. Four weeks after PQ administration, mice were fed and then anesthetized by an intraperitoneal injection of ketamine (60 mg/kg, Renfu Pharmaco, Yichang, China). Transthoracic echocardiographic images were obtained using an 18-MHz probe and the rodent mode (Vivid 7; GE Vingmed, Horten, Norway). The images were interpreted by an experienced echocardiographer blinded to the experiment. M-mode images were obtained in the left ventricular papillary short axis view. Left ventricular internal dimension of diastole (LVIDd), left ventricular internal dimension of systole (LVIDs), heart rate (HR), and ejection fraction (EF) were measured as a baseline (T0) before sevoflurane administration.

Sevoflurane treatment experiment

Mice in the WT-pulmonary fibrosis group, WT group, and $TLR4^{-/-}$ group were secured with a 20-gauge catheter tracheotomy tube after an intraperitoneal injection with ketamine and they were ventilated by a constant volume-cycled rodent ventilator (model 683, Harvard Apparatus, Holliston, MA, USA). The tidal volume was set at 5 mL/kg with respiratory rate of 130–140 /min. The mice were placed on the homoeothermic blanket to maintain a body temperature of 38°C. HR and EF were measured at 1 h (T1) and 2 h (T2) after exposure to 2% sevoflurane. Echocardiographic data were obtained while mechanical ventilation was suspended. All mice were sacrificed and hearts were harvested as described above.

Anti-inflammation experiment

Pulmonary fibrosis WT mice were given the TNF-α inhibitor lenalidomide (50 mg/kg/d, Selleck Chemicals LLC, Houston, TX, USA) intragastrically for three days, while the control group was treated with 1% dimethyl sulfoxide (DMSO). After three days, HR and EF were measured by echocardiogram before and after inhalation of sevoflurane at T0, T1, and T2.

Histopathological study

Mice were euthanized under deep anesthesia and blood was taken from the heart. The lung and heart were then removed. Tissue from the lung and heart, for histopathological analysis, was fixed in 4% paraformaldehyde and tissue for assessment of hydroxyproline content or extraction of protein was frozen in liquid nitrogen and stored at −80°C.

The lung and heart were embedded in paraffin blocks and cut into 5 -μm sections that were stained by hematoxylin and eosin (H&E) or Masson trichrome. Stained sections were assessed using a computerized morphometric system (Qwin, Leica, Wetzlar, Hessen, Germany).

Measurement of hydroxyproline content

Hydroxyproline content was measured using a hydroxyproline measurement kit (Jiancheng Company, Nanjing, China) according to the manufacturer's protocol. Briefly, lung tissues of six mice in each group were weighed and diluted in 1 mL of hydrolysate. Samples were then hydrolyzed at 95°C for 20 min. Reagent I (0.5 mL) was added to a blank tube, standard tube, and sample tube and the mixture was incubated for 10 min; 0.5 mL of Reagent II was added to the above tubes and incubated for 5 min; 0.5 mL of Reagent III was added to the above tubes and incubated at 60°C for 15 min; the mixture was centrifuged at 3500 *g* for 10 min and the supernatant was collected. Absorbance was measured at 550 nm wavelength using an Infiniti M200 (Tecan Group Ltd., Männedorf, Switzerland).

Western blot analysis

Sevoflurane's effects on TLR4 and iNOS expression in cardiomyocytes in the control and pulmonary fibrosis groups were analyzed by western blot. Total protein extracted from heart tissue was lysed on ice for 10 min by RIPA lysis buffer containing a 1:100 dilution of phenylmethanesulfonyl fluoride (Beyotime, Shanghai, China). The tissues were then sonicated and the crude extracts were centrifuged at 12,000 *g* for

10 min at 4°C. The concentration of protein was detected with a BCA Protein Assay Kit (Beyotime, Shanghai, China). Fifty micrograms of lysates were loaded onto a 10% SDS-PAGE gel and then transferred to polyvinylidene fluoride membranes (Merck Millipore, Billerica, MA, USA). After membranes were blocked with 5% non-fat milk in Tris-buffered saline plus 0.05% Tween 20 (pH = 7.5) for 1 h, they were probed overnight at 4°C with primary rabbit polyclonal anti-TLR4 (1:500; Abcam, Cambridge, MA, USA), rabbit polyclonal anti-iNOS (1:10, Abcam, Cambridge, MA, USA), rabbit monoclonal anti-GAPDH (1:1000, Cell Signal Technology, Danvers, MA, USA). Membranes were incubated with corresponding secondary antibodies, horseradish peroxidase-conjugated goat anti-rabbit antibodies (both 1:4000; ComWin Biotech, Beijing, China) at room temperature for 2 h. The bands were visualized by a super-enhanced chemiluminescent detection reagent (Merck Millipore, Billerica, MA, USA). Image Pro Plus 6.0 software was used for densitometry analysis. The protein content was normalized to the standardized GAPDH levels.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA). The values were expressed as means ± standard deviation (SD). Analysis of variance and a Student Newman–Keuls post hoc test was performed to determine the statistical significance ($P < 0.05$) of differences between groups.

Results

Pulmonary fibrosis and myocardial damage

All mice survived the PQ treatment. Compared with saline-treated mice, the PQ administration was associated with a significantly lower body weight in WT mice. Besides, the body weight of PQ-treated WT mice was lower than PQ-treated TLR4$^{-/-}$ mice (Table 1).

There were no significant differences in geometric or functional parameters measured by echocardiography between WT and TLR4$^{-/-}$ mice. After PQ treatment, LVIDd, LVIDs, and HR increased, and EF decreased in WT mice. However, these parameters did not change significantly in TLR4$^{-/-}$ mice. When compared with PQ-treated WT mice, LVIDd, LVIDs, and HR are lower and EF are higher in PQ-treated TLR4$^{-/-}$ mice (Table 1).

Histopathological changes in lung and heart tissue

H&E staining in the lung demonstrated that PQ administration induced inflammatory cell infiltration into the lung when compared with normal saline administration. TLR4$^{-/-}$ mice treated with PQ showed less change in morphology and fewer inflammatory cell infiltration into the lung when compared with PQ-treated WT mice (Fig. 1a–d). Masson staining showed a higher degree of fibrosis in WT mice than in TLR4$^{-/-}$ mice after PQ treatment (Fig. 1e–h). H&E staining in the heart showed that only part of the myocardial interstitial was infiltrated by inflammatory cells in WT mice that developed pulmonary fibrosis; the other three groups were normal (Fig. 1i–l). Masson staining of the right ventricle wall demonstrated that myocardial injury appeared in WT mice exhibiting myocardial interstitial fibrosis (Fig. 1m–p). Therefore, the degree of lung and heart inflammatory infiltration and injury was worse in WT mice than in TLR4$^{-/-}$ mice four weeks after PQ injection.

Measurement of hydroxyproline content

PQ significantly upregulated the hydroxyproline content in WT mice, whereas it did not change the levels in TLR4$^{-/-}$

Table 1. Characters and echocardiographic parameters of WT and TLR4$^{-/-}$ mice exposed to PQ or saline.

Parameter	WT	WT-PF	TLR4$^{-/-}$	TLR4$^{-/-}$PF
BW (g)	26.67 ± 0.64	24.54 ± 0.80*	26.02 ± 1.47	27.11 ± 1.79#
HW (mg)	115.71 ± 9.76	104.29 ± 7.87	118.57 ± 16.76	105.71 ± 13.97
LW (mg)	168.33 ± 28.28	153.33 ± 19.66	186.67 ± 17.51	171.67 ± 18.35
HW/BW (mg/g)	4.38 ± 0.39	4.28 ± 0.35	4.61 ± 0.59	3.93 ± 0.37
LW/BW (mg/g)	6.31 ± 1.05	6.27 ± 1.01	7.19 ± 0.80	6.35 ± 0.77
Hydroxyproline (ug/mg)	0.90 ± 0.08	1.03 ± 0.04*	0.85 ± 0.08	0.86 ± 0.04##
LVIDd (cm)	0.24 ± 0.030	0.31 ± 0.035***	0.24 ± 0.020	0.22 ± 0.014###
LVIDs (cm)	0.11 ± 0.036	0.17 ± 0.027***	0.099 ± 0.021	0.080 ± 0.012###
HR (bpm)	547 ± 23	611 ± 23**	564 ± 26	578 ± 28#
EF (%)	92.3 ± 3.5	77.2 ± 3.0***	93.4 ± 2.8	93.7 ± 2.0###

Values are presented as mean ± SD (n = 6 for each group).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, when compared with group WT; #$P < 0.05$, ###$P < 0.001$, when compared with group WT-PF.
BW, body weight; HW, heart weight; LW, lung weight; LVIDd, left ventricular internal dimension diastole; LVIDs, left ventricular internal dimension systole; HR, heart rate; EF, ejection fraction; WT, WT mice after a 28-day exposure to saline; WT-PF, WT mice after a 28-day exposure to paraquat; TLR4$^{-/-}$, TLR4$^{-/-}$ mice after a 28-day exposure to saline; TLR4$^{-/-}$PF, TLR4$^{-/-}$ mice after a 28-day exposure to paraquat.

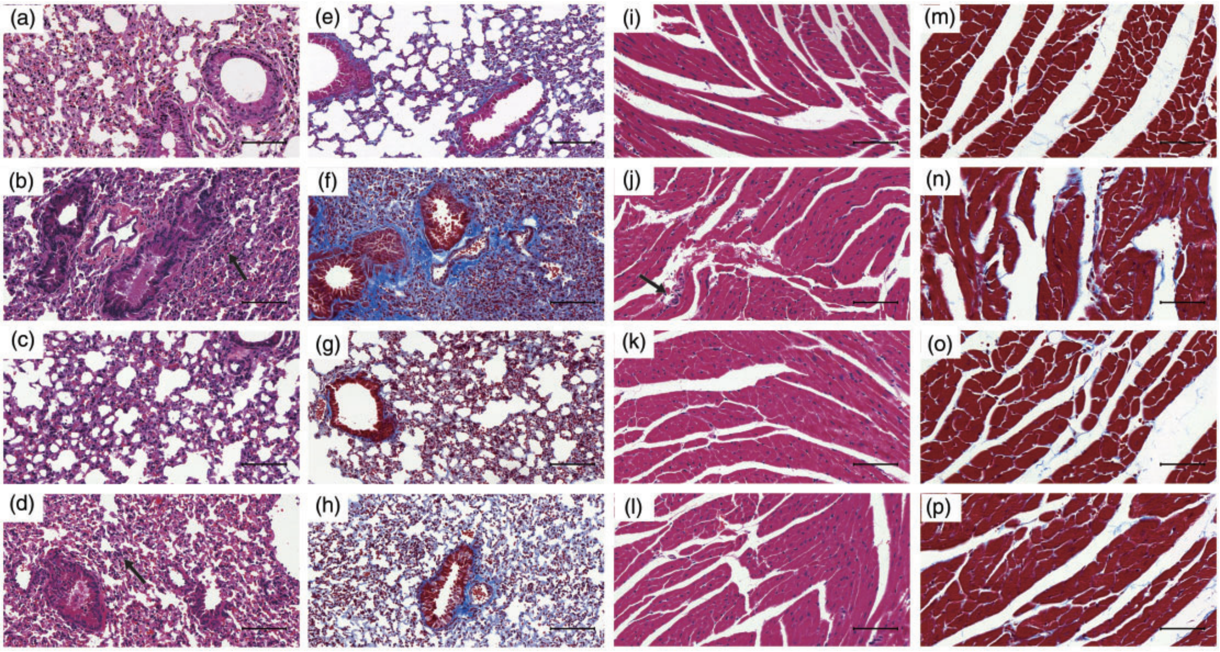

Fig. 1. Histopathological changes in WT and TLR4$^{-/-}$ mice after a 28-day exposure to PQ or saline. (a–d) H&E staining in the lung, scale bars, 100 μm; (e–h) Masson staining in the lung, scale bars, 100 μm; (i–l) H&E staining in the heart, scale bars, 50 μm; (m–p) Masson staining in the heart, scale bars, 50 μm; (a, e, i, m) WT group; (b, f, j, n) WT-pulmonary fibrosis group; (c, g, k, o) TLR4$^{-/-}$ group; (d, h, l, p) TLR4$^{-/-}$pulmonary fibrosis group. Arrows in (b), (d), and (j) indicate areas of inflammatory cell infiltration. Photomicrographs are representative of n = 6 in each group.

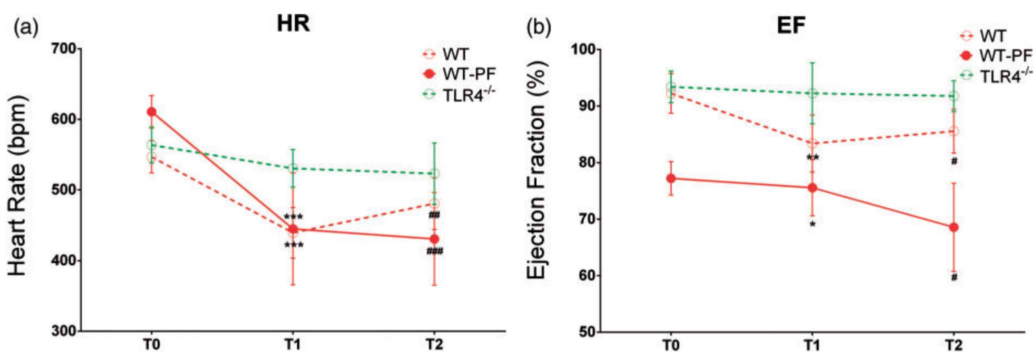

Fig. 2. The changes of HR and EF of each group after sevoflurane inhalation. WT: WT mice after a 28-d exposure to saline; WT-PF: WT mice after a 28-d exposure to paraquat; TLR4-/-: TLR4-/- mice after a 28-d exposure to saline. Values are presented as mean ± SD (n = 6 for each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, when T1 is compared with T0; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, when T2 is compared with T0.

mice. When compared with WT mice, the hydroxyproline content of the lung was less in TLR4$^{-/-}$ mice after PQ injection (Table 1).

Sevoflurane treatment and anti-inflammation experiment with the TNF-α inhibitor

After sevoflurane inhalation, HR and EF decreased significantly in WT mice and WT pulmonary fibrosis mice. TLR4$^{-/-}$ mice were more resistant to the effect of sevoflurane on HR and EF than that of WT mice. When compared with WT mice, WT pulmonary fibrosis mice suffered more decline in HR and EF after 2 h of sevoflurane inhalation (HR, WT pulmonary fibrosis mice vs. WT mice: 29.3% vs. 12.2%; EF, WT pulmonary fibrosis mice vs. WT mice: 11.2% vs. 7.2%) (Fig. 2).

Pulmonary fibrosis mice treated with lenalidomide showed a smaller decline in HR (30.5% vs. 22.3%) and had no change in EF after sevoflurane inhalation for 2 h when compared with mice treated with DMSO (Fig. 3).

Protein expression of TLR4 and iNOS in the heart

Protein levels of iNOS and TLR4 significantly increased after PQ treatment in WT mice ($P < 0.05$). After sevoflurane inhalation, iNOS and TLR4 expression were elevated in

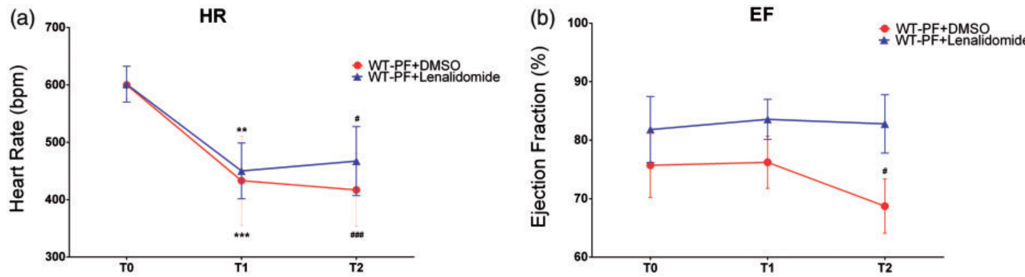

Fig. 3. The changes of HR and EF after sevoflurane inhalation in pulmonary fibrosis mice treated with lenalidomide or DMSO. WT-PF+Lenalidomide: pulomary fibrosis mice treated with lenalidomide; WT-PF+DMSO: pulomary fibrosis mice treated with DMSO. Values are presented as mean ± SD (n = 6 for each group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, when T1 is compared with T0; #$P < 0.05$, ###$P < 0.001$, when T2 is compared with T0.

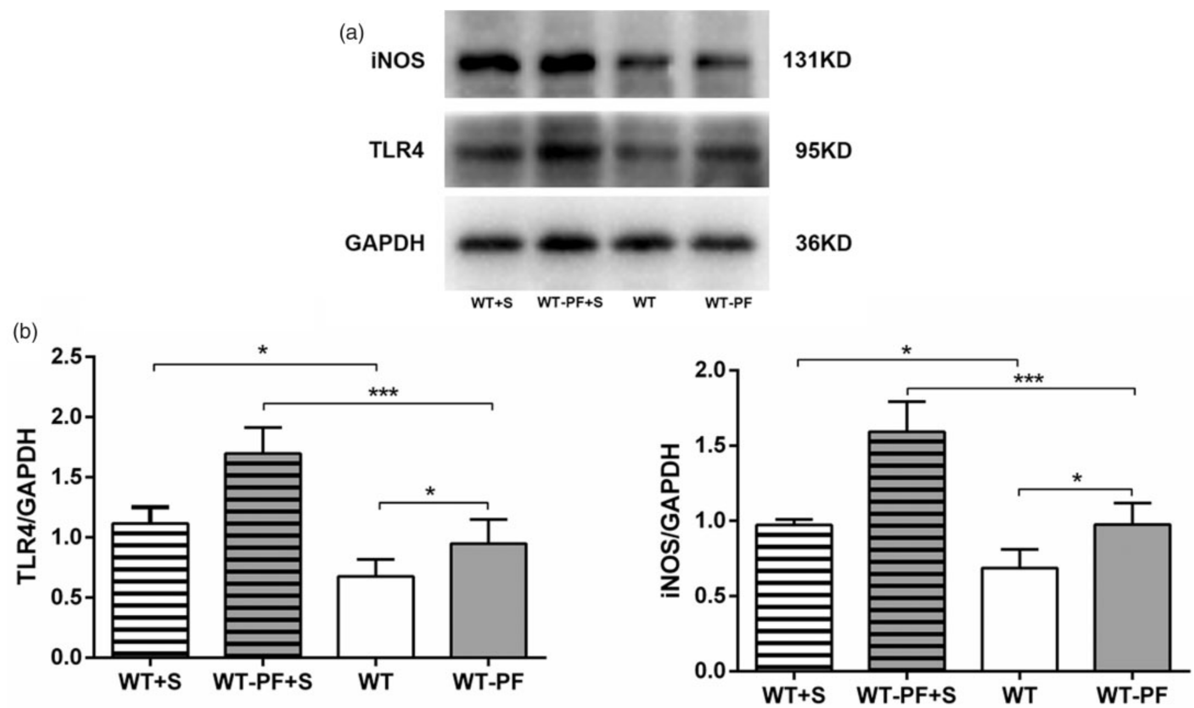

Fig. 4. The effects of sevoflurane on the expression of TLR4 and iNOS in mice hearts. (a) Representative images of iNOS (131 kD), TLR4 (95 kD), and GAPDH (36 kD) protein levels. (b) Relative amount of iNOS and TLR4. GAPDH was used as an internal control. Values are presented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 4 in each group.

both groups. Interestingly, PQ-treated WT mice exhibited an additional iNOS and TLR4 increase from their already higher baseline (Fig. 4).

Discussion

In this study, animal models of pulmonary fibrosis were induced by a single intraperitoneal injection of PQ, which is an irreversibly stable model.[16] Fibroblasts are activated and transformed into myofibroblasts under inflammatory stimulation. Myofibroblasts secrete large amounts of collagen and extracellular matrix,[17–19] leading to pulmonary fibrosis and myocardial injury.[20] The expression of TLR4 was increased in fibrotic tissues; however, the effect of regulating TLR4 expression on lung fibrosis was controversial.[21–26] In the present study, lung fibrosis was induced by injection of PQ successfully both in WT mice and TLR4$^{-/-}$ mice. We suspect TLR4$^{-/-}$ mice manifest reduced inflammatory infiltration and lung fibrosis after PQ administration based on qualitative histologic analyses, as shown by less histological evidence of inflammatory cellular infiltration and fibrosis, hydroxyproline content in the lung and heart.

In our previous study, left and right ventricular function were altered in rats with pulmonary arterial hypertension.[27,28] Studies have shown that acute high-dose PQ poisoning caused a decrease in cardiac contractility, an imbalance of intracellular Ca^{2+}, apoptosis acceleration, and mitochondrial damage.[29,30] In the present study, for WT mice after PQ treatment, morphological results showed that only part of the myocardial interstitial was infiltrated by inflammatory cells and part of the myocardium was injured. Echocardiographic images showed that LV volume increased and EF decreased in PQ-treated WT mice, whereas PQ did not induce obvious alternations in $TLR4^{-/-}$ mice. In addition, the protein level of TLR4 significantly increased after PQ treatment in WT mice. This may suggest that TLR4 could be associated with myocardial injury in pulmonary fibrosis mice by modulating the inflammatory response.

Studies have shown that 2.0% sevoflurane anesthesia resulted in apparent changes in microRNA expression in rat lungs, and some of the differentially expressed microRNAs were known to be involved in idiopathic pulmonary fibrosis.[31] Besides, sevoflurane protects the myocardium from ischemia-reperfusion injury through the TLR4 signaling pathway or downstream iNOS.[11–13,32] In this study, 2 h after sevoflurane administration, HR and EF decreased in WT pulmonary fibrosis mice, and TLR4 and iNOS expression levels were upregulated, indicating that inhibition of sevoflurane on cardiac function in pulmonary fibrotic mice may be related to TLR4 and iNOS. iNOS was located downstream of the TLR4/MyD88/NF-κB signaling pathway. Upregulation of iNOS may activate protein kinase G and its downstream cGMP, thereby reducing the sensitivity of the myofilament to Ca^{2+} and reducing cardiac contractility.[14] In the present study, WT pulmonary fibrosis mice exhibited a further iNOS and TLR4 increase from their already high baseline after 2 h of sevoflurane inhalation. In addition, WT mice suffered more decline in HR and EF after 2 h of sevoflurane inhalation, while, $TLR4^{-/-}$ mice were more resistant to the effect of sevoflurane on HR and EF than that of WT mice. This indicates that TLR4 and its downstream iNOS are related to sevoflurane's inhibition of cardiac function and that the deficiency of the TLR4 gene attenuated the sevoflurane's inhibitory effect on cardiac function.

In the TLR4/MyD88/NF-κB signaling pathway, TLR4 upregulates downstream NF-κB expression, thereby promoting the expression of TNF-α.[33] As a pro-inflammatory factor, TNF-α causes inflammatory reactions and promotes myocardial injury.[34] Studies have showed that TNF-α inhibitors such as lenalidomide can improve myocardial contractile dysfunction in obese mice.[15] In acute high-dose PQ exposure, myocardial injury and myocardial contractile dysfunction were associated with TLR4 as well as its downstream cytokine, TNF-α.[35] In this study, WT pulmonary fibrosis mice treated with lenalidomide showed less decline in HR and had no change in EF after 2 h of sevoflurane inhalation. Heart function was significantly optimized in WT pulmonary fibrosis mice by a reduction of the inflammatory response through inhibiting the secretion of TNF-α.

Study limitations

In this study, quantification of histological analyses was not acquired. We did not measure systemic blood pressure of the mice except for echocardiography. We also did not investigate the inflammation reaction in a pulmonary fibrosis model. How TLR4 mediates sevoflurane's inhibition of cardiac function in mice with pulmonary fibrosis still needs to be investigated.

Conclusion

Sevoflurane depresses cardiac function in mice with pulmonary fibrosis through the TLR4/iNOS pathway. Lenalidomide can attenuate the effect of sevoflurane on cardiac function in mice with pulmonary fibrosis.

Conflict of interest

The author(s) declare that there is no conflict interest.

Funding

This work was supported by a grant from The National Natural Science Foundation of China (no. 81370251).

References

1. Duffield JS, Lupher M, Thannickal VJ, et al. Host responses in tissue repair and fibrosis. *Annu Rev Pathol* 2013; 8: 241–276.
2. Raghu G, Amatto VC, Behr J, et al. Comorbidities in idiopathic pulmonary fibrosis patients: a systematic literature review. *Eur Respir J* 2015; 46(4): 1113–1130.
3. Ghatol A, Ruhl AP and Danoff SK. Exacerbations in idiopathic pulmonary fibrosis triggered by pulmonary and nonpulmonary surgery: a case series and comprehensive review of the literature. *LUNG* 2012; 190(4): 373–380.
4. Gay NJ and Gangloff M. Structure and function of Toll receptors and their ligands. *Annu Rev Biochem* 2007; 76: 141–165.
5. Dolan J, Walshe K, Alsbury S, et al. The extracellular leucine-rich repeat superfamily; a comparative survey and analysis of evolutionary relationships and expression patterns. *BMC Genomics* 2007; 8: 320.
6. Matsushima N, Tachi N, Kuroki Y, et al. Structural analysis of leucine-rich-repeat variants in proteins associated with human diseases. *Cell Mol Life Sci* 2005; 62(23): 2771–2791.
7. Takaesu G, Kishida S, Hiyama A, et al. TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Mol Cell* 2000; 5(4): 649–658.
8. Bassuoni AS and Amr YM. Cardioprotective effect of sevoflurane in patients with coronary artery disease undergoing vascular surgery. *Saudi J Anaesth* 2012; 6(2): 125–130.
9. Huseidzinovic I, Barisin S, Bradic N, et al. Early cardioprotective effect of sevoflurane on left ventricular performance during coronary artery bypass grafting on a beating heart: randomized controlled study. *Croat Med J* 2007; 48(3): 333–340.
10. Royse CF, Liew DF, Wright CE, et al. Persistent depression of contractility and vasodilation with propofol but not with sevoflurane or desflurane in rabbits. *Anesthesiology* 2008; 108(1): 87–93.
11. Zhang J, Zhang J, Yu P, et al. Remote ischaemic preconditioning and sevoflurane postconditioning synergistically protect rats from myocardial injury induced by ischemia and

reperfusion partly via inhibition TLR4/MyD88/NF-kappaB signaling pathway. *Cell Physiol Biochem* 2017; 41(1): 22–32.
12. Ma LL, Zhang FJ and Yan M. [Sevoflurane preconditioning produces delayed cardioprotection effect through up-regulation of inducible nitric oxide synthase in rats]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2012; 41(5): 553–558.
13. Kaneda K, Miyamae M, Sugioka S, et al. Sevoflurane enhances ethanol-induced cardiac preconditioning through modulation of protein kinase C, mitochondrial KATP channels, and nitric oxide synthase, in guinea pig hearts. *Anesth Analg* 2008; 106(1): 9–16.
14. Layland J, Li JM and Shah AM. Role of cyclic GMP-dependent protein kinase in the contractile response to exogenous nitric oxide in rat cardiac myocytes. *J Physiol* 2002; 540(Pt 2): 457–467.
15. Li L, Hua Y, Dong M, et al. Short-term lenalidomide (Revlimid) administration ameliorates cardiomyocyte contractile dysfunction in ob/ob obese mice. *Obesity (Silver Spring)* 2012; 20(11): 2174–2185.
16. Moore BB and Hogaboam CM. Murine models of pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2008; 294(2): L152–L160.
17. Nusslein-Volhard C and Wieschaus E. Mutations affecting segment number and polarity in Drosophila. *Nature* 1980; 287(5785): 795–801.
18. Derynck R and Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; 425(6958): 577–584.
19. Bhattacharyya S, Kelley K, Melichian DS, et al. Toll-like receptor 4 signaling augments transforming growth factor-beta responses: a novel mechanism for maintaining and amplifying fibrosis in scleroderma. *Am J Pathol* 2013; 182(1): 192–205.
20. Chen M, Cheung FW, Chan MH, et al. Protective roles of Cordyceps on lung fibrosis in cellular and rat models. *J Ethnopharmacol* 2012; 143(2): 448–454.
21. Yoshizaki A, Iwata Y, Komura K, et al. CD19 regulates skin and lung fibrosis via Toll-like receptor signaling in a model of bleomycin-induced scleroderma. *Am J Pathol* 2008; 172(6): 1650–1663.
22. Li XX, Jiang DY, Huang XX, et al. Toll-like receptor 4 promotes fibrosis in bleomycin-induced lung injury in mice. *Genet Mol Res* 2015; 14(4): 17391–17398.
23. Tao L, Yang J, Cao F, et al. Mogroside IIIE, a novel anti-fibrotic compound, reduces pulmonary fibrosis through toll-like receptor 4 pathways. *J Pharmacol Exp Ther* 2017; 361(2): 268–279.
24. Yang HZ, Wang JP, Mi S, et al. TLR4 activity is required in the resolution of pulmonary inflammation and fibrosis after acute and chronic lung injury. *Am J Pathol* 2012; 180(1): 275–292.
25. Liang J, Zhang Y, Xie T, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. *Nat Med* 2016; 22(11): 1285–1293.
26. Ebener S, Barnowski S, Wotzkow C, et al. Toll-like receptor 4 activation attenuates profibrotic response in control lung fibroblasts but not in fibroblasts from patients with IPF. *Am J Physiol Lung Cell Mol Physiol* 2017; 312(1): L42–L55.
27. Qin G, Luo H, Yin X, et al. Effects of sevoflurane on hemodynamics and inducible nitric oxide synthase/soluble guanylate cyclase signaling pathway in a rat model of pulmonary arterial hypertension. *Anesth Analg* 2017; 125(1): 184–189.
28. Wang L, Luo H, Qin G, et al. The impact of sevoflurane on coupling of left ventricular to systemic vasculature in rats with chronic pulmonary hypertension. *J Cardiothorac Vasc Anesth* 2017; 31(6): 2027–2034.
29. Wang J, Lu S, Zheng Q, et al. Cardiac-specific knockout of ETA receptor mitigates paraquat-induced cardiac contractile dysfunction. *Cardiovasc Toxicol* 2016; 16(3): 235–243.
30. Dong X, Wang R, Xu X, et al. Influence of paraquat poisoning on the expression of toll-like receptor 4 in myocardial damage following in mice and its significance. *Journal of China Medical University* 2015; 44(10): 891–896, 900.
31. Tanaka S, Ishikawa M, Arai M, et al. Changes in microRNA expression in rat lungs caused by sevoflurane anesthesia: a TaqMan(R) low-density array study. *Biomed Res* 2012; 33(5): 255–263.
32. Zhang FJ, Ma LL, Wang WN, et al. Hypercholesterolemia abrogates sevoflurane-induced delayed preconditioning against myocardial infarct in rats by alteration of nitric oxide synthase signaling. *Shock* 2012; 37(5): 485–491.
33. Akira S, Uematsu S and Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; 124(4): 783–801.
34. Zhang J, Cao Y, Gao X, et al. Lipopolysaccharide acutely suppresses right-ventricular strain in rats with pulmonary artery hypertension. *Pulm Circ* 2018; 8(1): 1–7.
35. Ke JJ, Yu FX, Rao Y, et al. Adenosine postconditioning protects against myocardial ischemia-reperfusion injury though modulate production of TNF-alpha and prevents activation of transcription factor NF-kappaB. *Mol Biol Rep* 2011; 38(1): 531–538.