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Peroxiredoxin 6 knockout aggravates cecal ligation and puncture-induced acute lung injury

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ABSTRACT

Background: The aim of present study was to investigate the effects and mechanisms of *peroxiredoxin* (*Prdx*) *6* on cecal ligation and puncture (CLP) induced acute lung injury (ALI) in mice.

Methods: The cecal of male *Prdx 6* knockout and wildtype C57BL/6J mice were ligated and perforated. Stool was extruded to ensure wound patency. Two hours, 4 h, 8 h and 16 h after stimulation, the morphology, wet/dry ratio, protein concentration in bronchial alveolar lavage fluid (BALF) were measured to evaluate lung injury. Myeloperoxidase (MPO) activity, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), total superoxide dismutase (SOD), xanthine oxidase (XOD), CuZn-SOD, total anti-oxidative capability (TAOC), glutathione peroxidase (GSH-PX), catalase (CAT) in lungs were measured by assay kits. The mRNA expression of lung tumor necrosis factor (TNF-α), interleukin (IL)-1β, and matrix metalloproteinases (MMP) 2 and 9 were tested by real-time RT-PCR. The nuclear factor (NF)-κB activity was measured by TransAM kit.

Results: CLP-induced ALI was characterized by inflammation in morphology, increased wet/dry ratio, elevated protein concentration in BALF and higher level of MPO activity. The levels of H_2O_2, MDA, and XOD were significantly increased and SOD, CuZn-SOD, GSH-PX, CAT, and T-AOC were significantly decreased in lungs after CLP. The activity of NF-κB was significantly increased and subsequently, the mRNA expression of TNF-α, IL-1β and MMP2 and MMP9 were significantly increased after CLP. Those above injury parameters were more severe in *Prdx 6* knockout mice than those in wildtype mice.

Conclusions: Prdx 6 knockout aggravated the CLP induced lung injury by augmenting oxidative stress, inflammation and matrix degradation partially through NF-κB pathway.

1. Introduction

Acute lung injury/acute respiratory distress syndrome (ALI[2]/ARDS[3]) is a clinical syndrome characterized by respiratory stress, diffuse lung infiltration and acute progressive respiratory failure [1]. It has been shown that oxidative stress plays important roles in the pathogenesis of ALI. Reactive oxygen species (ROS)[4] can lead to lung injury through lipid and protein peroxidation, nucleic acid injury, and transcriptional factor activation [2].

Peroxiredoxins (*Prdx*)[5] are a recently discovered non-selenium dependent peroxidases superfamily in mammalian animals over 10 years [3]. *Prdx 6* is one of their subtypes and highly expresses in the lung, particularly in type II alveolar cells and Clara cells [4]. *Prdx 6* plays important roles in scavenging pulmonary ROS and in the phospholipid metabolism. It has been reported that *Prdx 6* could protect the lung from oxidative stress caused by hyperoxia and parquet [5,6]. Our team previously demonstrated that *Prdx 6* could also protect the lung against LPS-induced injury [7]. It remains unclear whether *Prdx 6* could protect sepsis-induced ALI. Still, a lot more need to be understood.

ALI/ARDS usually occurs after serious infection, shock, trauma and

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[2] ALI: acute lung injury.
[3] ARDS: acute respiratory distress syndrome.
[4] ROS: reactive oxygen species.
[5] Prdx: peroxiredoxins.

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burn [1]. The pathogenesis of ALI/ARDS is still not totally clear. Various kinds of animal models are used for basic research, among which the cecal ligation and puncture (CLP)[6] model is a putative animal model ideal for clinical sepsis [8,9]. In the CLP model, systemic inflammatory reaction can be observed following typical trauma and infection [10–12]. The aim of the study was to elucidate the roles and mechanisms of *Prdx 6* in CLP-induced lung injury.

2. Material and methods

2.1. Animals

Prdx 6 knockout (−/−) and littermate wildtype C57BL/6J male mice (8–12 weeks of age, male, weight 20 ± 2 g) were used in this study. *Prdx 6* (−/−) mice were generously presented by Professor Aron B. Fisher from the University of Pennsylvania Medical Center. C57BL/6J male mice were purchased from Shanghai SLAC laboratory animal Co. Ltd. The animal protocols were approved by the Animal Care Committee of Fudan University. Mice were equally divided into four groups of *Prdx 6* (−/−) CLP group, C57BL/6J CLP group, *Prdx 6*(−/−) sham group and the C57BL/6J control group.

2.2. Mouse cecal ligation and puncture model

The mice were given ketamine (80 mg/kg) and analgesia with xylazine (30 mg/kg) intraperitoneally. Under sterile conditions, the cecum was ligated with 3-0 silk sutures about 10 mm proximal to the cecal pole and then punctured with an 18-gauge needle (Becton, Dickinson and Company, USA) [13]. A small amount of stool was squeezed out of the holes. Then, the cecum was repositioned and the laparotomy was closed with 4-0 silk sutures. The mice were returned to their cages with free access to food and water. Two hours, 4 h, 8 h and 16 h after cecal ligation and puncture surgery, mice were anesthetized with pentobarbital sodium (50 mg/kg) for further tests and then sacrificed.

2.3. Pulmonary histopathology

Lungs were obtained and fixed overnight with 10% formaldehyde, subjected to dehydration using an ethanol gradient, treated with xylenes and imbedded in paraffin. The tissues mounted in paraffin were sectioned, stained with hematoxylin and eosin and observed via light microscopy. Infiltration by inflammatory cells in the lung alveolar space and obvious interstitial edema were observed.

2.4. Lung injury measurement

Four hours after CLP, entire lungs were harvested for lung wet/dry ratio (W/D)[7] measurement to assess pulmonary edema [14]. Bronchoalveolar lavage was performed with intratracheal instillation of 0.9% saline (0.5 mL) into the lungs in situ gently and repeated for three times. The bronchoalveolar lavage fluid (BALF)[8] was centrifuged for 10 min at 1200 RPM. The supernatants were then collected and stored at −80 °C. Protein concentration in BALF was measured via the Micro BCA Protein Assay Kit (Pierce, MA). The myeloperoxidase (MPO)[9] activity in lung was measured with an assay kit from Nanjing Jiancheng (Nanjing, China) as described previously [15].

2.5. Oxidative stress test

The levels of hydrogen peroxide (H_2O_2)[10], malondialdehyde (MDA)[11], total superoxide dismutase (SOD)[12], CuZn-SOD, xanthine oxidase (XOD)[13], glutathione peroxidase (GSH-PX)[14], catalase (CAT)[15] and total anti-oxidative capability (TAOC)[16] in lungs were tested using commercial enzymatic kits from Nanjing Jiancheng (Nanjing, China) as described previously [15].

2.6. NF-κB activity measurement

Nuclear protein from the left lung was extracted by extraction kit (Active Motif, Carlsbad, CA, USA). Nuclear factor (NF)-κB[17] DNA binding activity was then measured using the non-radioactive NF-κB p65 transcription factor activity kit (Active Motif, Carlsbad, CA) [7].

2.7. Real-time reverse quantitative polymerase chain reaction (RT-PCR) of mRNA expressions

Total RNA from lungs was isolated (Trizol Reagent, Invitrogen, US) and reversely transcribed into complementary deoxyribonucleic acid (cDNA)[18] (SYBR PrimeScript RT-PCR Kit, TaKaRa, Japan). Then, 40 ng cDNA was used as a template for the operation of real time RT-PCR according to manufacturer's instructions (Select SYBR Premix Ex Taq™ II, TaKaRa, Japan). The tumor necrosis factor (TNF-α)[19], interleukin (IL)-1β[20], matrix metalloproteinases (MMP)[21] 2 and 9 and β-actin gene primers were designed using the mouse genome sequence published in GenBank and synthesized by TaKaRa (TaKaRa, Shanghai). The primer sequences were as follows: IL-1β forward: 5′-CTACAGGCTCCGAGATGAACAAC-3′; IL-1β reverse: 5′-CTACAGGCTCCGAGATGAACAAC-3′; TNF-α forward: 5′-ATCCGCGACGTGGAACTG-3′; TNF-α reverse: 5′-ACCGCCTGGAGTTCTGGAA-3′; MMP2 forward: 5′-CCCTCAAGAAGATGCAGAAGTTC-3′; MMP2 reverse: 5′-GCTTCCGCATGGTCTCGAT-3′; MMP9 forward: 5′-CCAAGGGTACAGCCTGTTCCT-3′; MMP9 reverse: 5′-GCACGCTGGAATGATCTAAGC-3′; β-actin forward: 5′-ACGGCCAGGTCATCACTATTG-3′ and β-actin reverse: 5′-TGGATGCCACAGGATTCCAT-3′. The amplification cycling reactions (40 cycles) were performed as follows: 15 s at 95 °C and 1 min at 60 °C. The relative quantification concentrations of mRNA expressions in the samples were calculated according to the standard curve and analyzed by Rotor-Gene 3000 Sequence Detection System at the comparative threshold cycle ($2^{-\Delta\Delta ct}$).

3. Calculation

Data was presented as mean ± standard deviation (SD). The homogeneity variance was compared in every group. Normally-distributed continuous variables of multiple groups were compared by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. *t*-Test was used to analyze difference between two groups of mice. All statistical assessments were evaluated at the 0.05 level of significant difference. Statistical significance was analyzed with SPSS 19 (SPSS Inc., Chicago, IL, USA).

[6] CLP: cecal ligation and puncture.
[7] W/D: wet/dry ratio.
[8] BALF: bronchoalveolar lavage fluid.
[9] MPO: myeloperoxidase.
[10] H_2O_2: hydrogen peroxide.
[11] MDA: malondialdehyde.
[12] SOD: superoxide dismutase.
[13] XOD: xanthine oxidase.
[14] GSH-PX: glutathione peroxidase.
[15] CAT: catalase.
[16] TAOC: total anti-oxidative capability.
[17] NF-κB: nuclear factor-κB.
[18] cDNA: complementary deoxyribonucleic acid.
[19] TNF-α: tumor necrosis factor-α.
[20] IL-1β: interleukin-1β.
[21] MMP: matrix metalloproteinases.

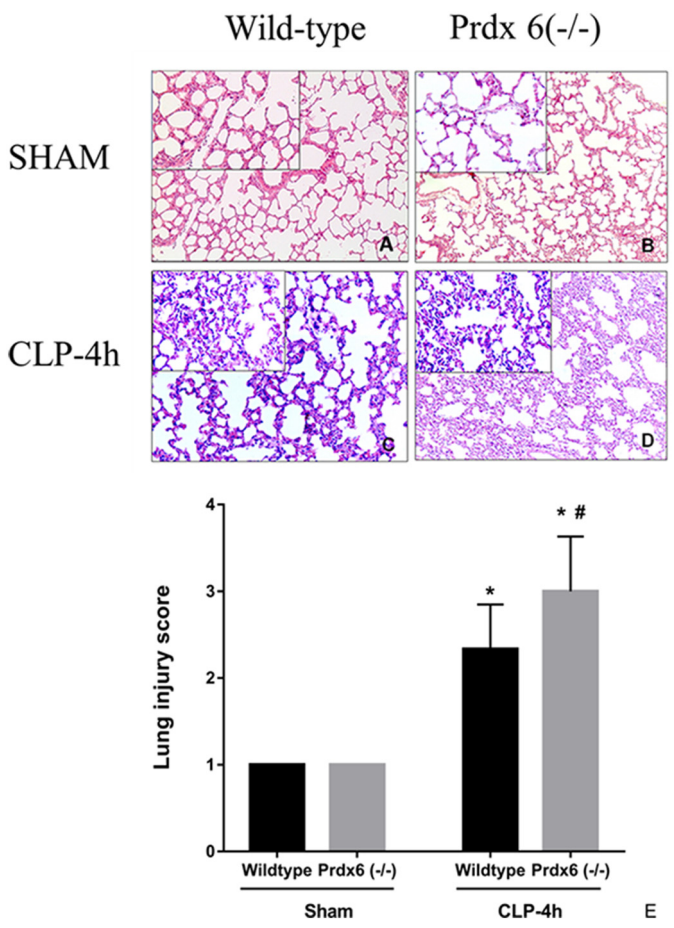

Fig. 1. Effects of cecal ligation and puncture (CLP) on pulmonary histopathology and lung injury score.
Peroxiredoxin (*Prdx*) *6*(−/−) and wild-type (WT) mice were subjected to cecum ligation and puncture for 4 h. Representative histology pictures of CLP-induced acute lung injury in WT and *Prdx 6* (−/−) mice. (A and B) Normal pulmonary alveoli structures in the sham group of WT and *Prdx 6* (−/−) mice (n = 5). (C) In CLP WT group, the significant neutrophils infiltration, pulmonary interstitial edema, thickened alveolar septal (n = 6). (D) Much aggravated lung injury in *Prdx 6* (−/−) mice (n = 6). (E) The lung injury scores were significantly increased in CLP groups as compared to that in sham group. *Prdx 6* knockout significantly increased lung injury score after CLP. Tissue sections were stained with hematoxylin and eosin (×200). Values are expressed as means ± SD. *p < 0.05 as compared with vehicle-challenged mice; #p < 0.05 as compared with CLP-challenged WT mice.

4. Results

4.1. The pathologic changes of lung tissue

The pathological changes of lung tissues were demonstrated in Fig. 1. No apparent inflammatory changes in lungs were observed in the control group of wildtype mice and *Prdx 6* (−/−) mice, with complete structures of pulmonary alveoli, no edema fluid in alveolar space, no inflammatory cells infiltration in the lung parenchyma and alveoli space (Fig. 1a and b). CLP-induced lung injury was characterized by pulmonary interstitial edema and thickened alveolar septal, edema fluid and infiltration of neutrophils in the alveolar spaces as observed via pulmonary histopathology (Fig. 1c). The degrees edema and inflammatory cells infiltration were more severe in *Prdx 6* (−/−) mice as compared to wildtype mice (Fig. 1d).

4.2. Lung injury score

Lung injury was scored as the followings: (i) pulmonary congestion, (ii) pulmonary haemorrhage, (iii) infiltration of neutrophils into the airspace or the vessel wall, and (iv) thickness of the alveolar wall. Each item was graded according to a five-point scale: 0, minimal (little) damage; 1, mild damage; 2, moderate damage; 3, severe damage; and 4, maximal damage. The score was significantly higher in CLP groups as compared to that in sham group. *Prdx 6* deficiency significantly augmented CLP increased lung injury score (Fig. 1e).

4.3. The pulmonary edema and pulmonary capillary permeability

The pulmonary edema was measured by wet to dry ratio. Compared to sham group, the wet to dry ratio in wildtype mice was increased but not significantly after CLP. The W/D ratio presented an increasing trend after CLP, reached the peak at 8 h after CLP and then declined nearly to the level of sham group at 16 h after CLP. However, only the W/D ratio at 4 h and 8 h after CLP in *Prdx 6* (−/−) mice was significantly higher than those of sham group and corresponding wildtype mice (Fig. 2a). In wildtype mice, the protein concentration in BALF was only significantly higher than that of sham group at 4 h after CLP. Then it dropped nearly to the degree of sham group. But in *Prdx 6* (−/−) mice, the protein concentration in BALF started to be significantly elevated 2 h after CLP as compared to those of sham group and corresponding wildtype mice. It sustained at approximately high level at 4 h and 8 h after CLP. The level at 16 h after CLP was still higher but not significantly than those of sham group and corresponding wildtype mice (Fig. 2b).

4.4. The MPO activity in lung tissue

The MPO activity was not significantly changed in *Prdx 6* deficiency mice as compared with wildtype mice. Four hours after CLP, the MPO activity was significantly increased in wildtype mice as compared with those of sham group. The MPO activity presented increasing trend 16 h after CLP but not significantly higher as compared to that of wildtype mice at 4 h after CLP. The MPO activity in *Prdx 6* (−/−) mice was significantly increased in a time dependent after CLP and was all markedly higher than that of wildtype mice at all time points (Fig. 2c).

4.5. The oxidative stress in lungs

CLP-induced lung injury was characterized by significantly enhanced oxidative stress. The levels of H_2O_2 (Fig. 3a), as the main ROS component, were significantly increased in a time dependent manner in *Prdx 6* (−/−) mice as compared to those of sham group and corresponding wildtype mice. The level in wildtype mice was only significantly increased 4 h after CLP as compared to that of sham group and then dropped back to nearly normal level 16 h after CLP. The levels of MDA (Fig. 3b), as the indicator for lipid peroxidation, were significantly increased in a time dependent manner in wildtype and *Prdx 6* (−/−) mice as compared to those of sham group. Only the MDA level at 4 h after CLP in *Prdx 6* (−/−) mice was significantly higher than that of corresponding wildtype mice. The levels of TSOD, CuZn-SOD, GSH-PX, CAT and T-AOC (Fig. 3c–g) were significantly decreased in wildtype and *Prdx 6* (−/−) mice as compared to those of sham group at both 4 h and 16 h after CLP. In *Prdx 6* (−/−) mice, the CuZn-SOD level at 4 h after CLP, the GSH-PX, CAT and T-AOC levels at 16 h after CLP, were significantly lower than those of corresponding wildtype mice.

4.6. NF-κB DNA binding activity

As our result showed the lung injury was significantly severe at 4 h after CLP, we chose to test NF-κB DNA binding activity in lungs at this time point. The NF-κB DNA binding activities in wildtype and *Prdx 6*

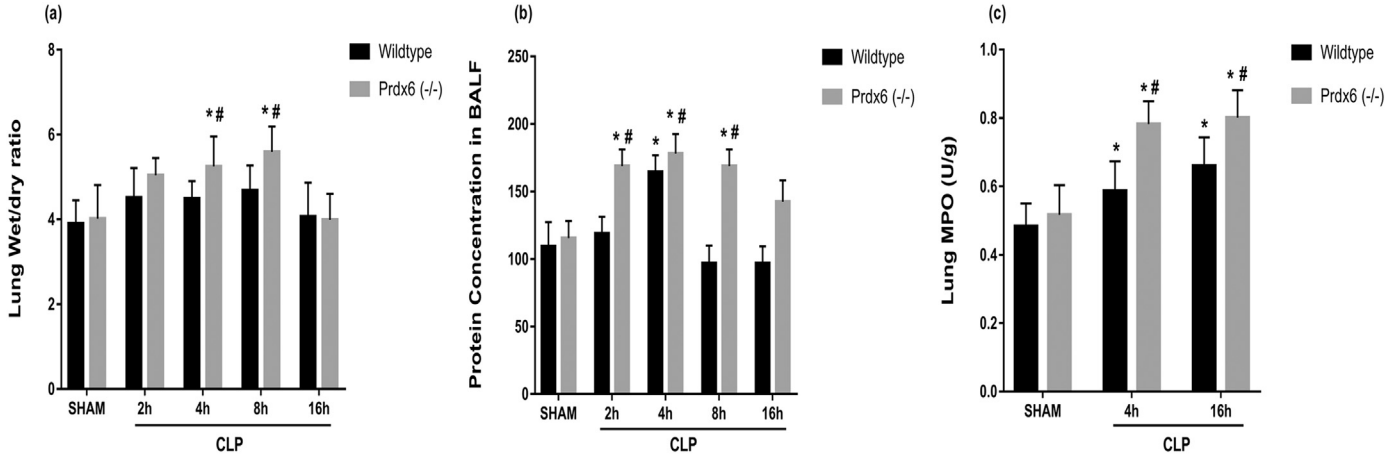

Fig. 2. The measurement of pulmonary edema and pulmonary capillary permeability.
Peroxiredoxin (*Prdx*) *6*(−/−) and wild-type (WT) mice were subjected to cecal ligation and puncture (CLP) for 2 h, 4 h, 8 h, and 16 h. (A) Wet to dry ratio was measured in each group as the indicator of pulmonary edema. Lung W/D ratio in *Prdx6* (−/−) mice was significantly increased at 4 h and 8 h after CLP as compared to those of WT mice. (n = 6 per group). (B) The total protein concentrations in bronchoalveolar lavage fluid (BALF) were determined in each group. The total protein concentrations in BALF in *Prdx6* (−/−) mice were significantly increased at 2 h, 4 h and 8 h after CLP as compared to those of WT mice. (n = 6 per group). (C) Myeloperoxidase (MPO) activity in lungs was measured in each group. MPO activity was significantly higher at 4 h and 16 h in *Prdx6* (−/−) mice after CLP than those of WT mice. (n = 6 per group). Values are expressed as means ± SD. *p < 0.05 as compared with vehicle-challenged mice; #p < 0.05 as compared with CLP-challenged WT mice.

(−/−) mice were significantly increased after CLP challenge as compared to that of sham group. At the same time, the NF-κB DNA binding activity in *Prdx 6* (−/−) mice was much higher than that in corresponding wildtype mice (Fig. 4).

4.7. The cytokines expression

The mRNA levels of TNF-α and IL-1β were significantly increased from 4 h after CLP in wildtype mice compared with those of sham group. The mRNA levels reached the peaks after CLP at 4 h for TNF-α

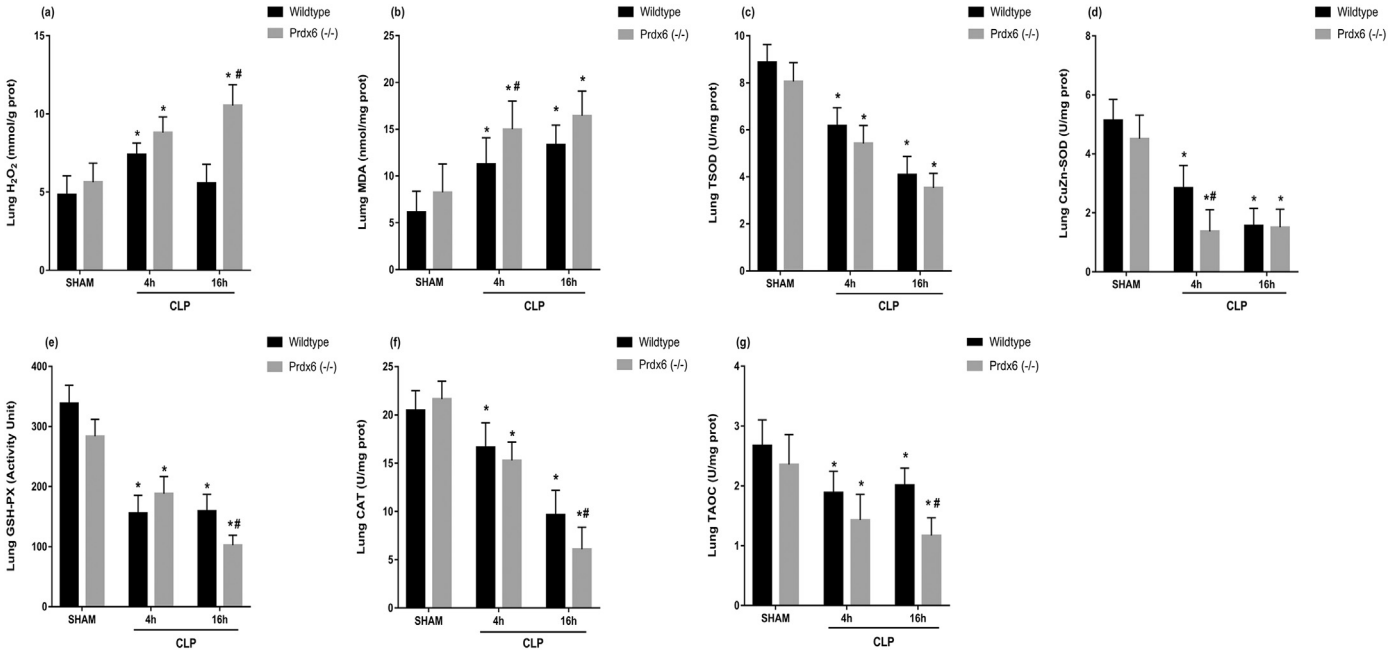

Fig. 3. Enhanced oxidative stress markers in *Peroxiredoxin* (*Prdx*) *6*(−/−) mice.
Prdx 6(−/−) and wild-type (WT) mice were subjected to cecal ligation and puncture (CLP) for 4 h and 16 h. (A) Levels of hydrogen peroxide H_2O_2. Levels of H_2O_2 were significantly increased at 16 h after CLP in *Prdx 6* (−/−) mice and markedly higher than those of WT mice. (B) Levels of malondialdehyde (MDA). Levels of MDA were significantly increased at 4 h after CLP in *Prdx 6* (−/−) mice and markedly higher than those of WT mice. (C) Levels of total superoxide dismutase (T-SOD). Levels of T-SOD were significantly decreased after CLP in a time dependent manner in *Prdx 6* (−/−) mice and markedly lower compared to those of sham group. (D) Levels of CuZn-SOD. Levels of CuZn-SOD were significantly decreased at 4 h after CLP in *Prdx 6* (−/−) mice and markedly lower than those of the corresponding WT mice. (E) Levels of glutathione peroxidase (GSH-PX). Levels of GSH-PX were significantly decreased at 16 h after CLP in *Prdx 6* (−/−) mice and markedly lower compared to those of the corresponding WT mice. (F) Levels of catalase (CAT). Levels of CAT were significantly decreased at 16 h after CLP in *Prdx 6* (−/−) mice and markedly lower compared to those of the corresponding WT mice. (G) Levels of total anti-oxidative capability (TAOC). Levels of TAOC were significantly decreased 16 h after CLP in *Prdx 6* (−/−) mice and markedly lower than those of WT mice. Values are expressed as means ± SD. *p < 0.05 as compared with vehicle-challenged mice; #p < 0.05 as compared with CLP-challenged WT mice (n = 6 in each group).

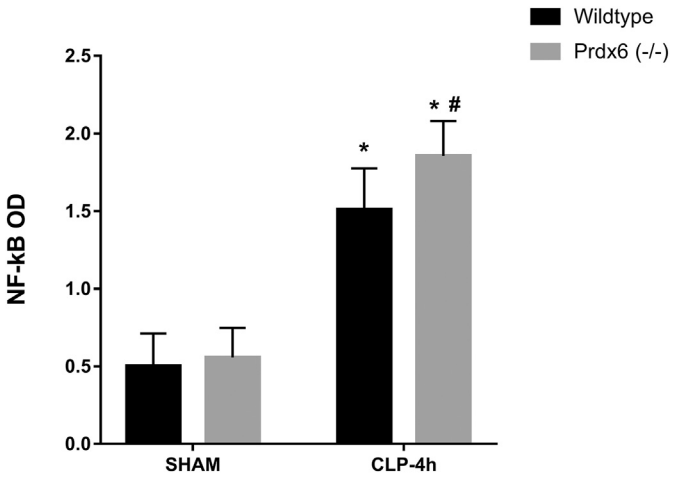

Fig. 4. The detection of nuclear factor (NF)-κB DNA binding activity.
Peroxiredoxin (*Prdx*) *6*(−/−) and wild-type (WT) mice were subjected to cecal ligation and puncture (CLP) for 4 h. The NF-κB DNA binding activity was tested as NF-κB OD. The NF-κB DNA binding activity was significantly higher at 4 h after CLP in *Prdx 6* (−/−) mice than those of sham group and corresponding WT mice. Values are expressed as means ± SD. *p < 0.05 as compared with vehicle-challenged mice; #p < 0.05 as compared with CLP-challenged WT mice (n = 6 in each group).

and at 8 h for IL-1β. Then, the mRNA levels of both TNF-α and IL-1β declined at 16 h after CLP but still significantly higher than those of sham. The mRNA levels of TNF-α and IL-1β in lungs of *Prdx 6* (−/−) mice were significantly increased in a time-dependent manner from 4 h after CLP and reached the peaks at 16 h after CLP. The mRNA levels of TNF-α and IL-1β in lungs of *Prdx 6* (−/−) mice were similar to those of wildtype mice at 4 h and 8 h time points but markedly higher than those of corresponding wildtype mice at 16 h (Fig. 5).

4.8. The MMPs expression

The mRNA level of MMP2 remained unchanged after CLP in wildtype mice. But it was significantly increased 2 h right after CLP challenge in a time dependent manner in *Prdx 6* (−/−) mice as compared to those of sham and corresponding wildtype mice. The mRNA level of MMP9 were significantly increased 8 h and 16 h after CLP in wildtype mice as compared to those of sham, which were much higher in *Prdx 6* (−/−) mice than those of sham and corresponding wildtype mice and started from 4 h after CLP (Fig. 6).

5. Discussion

Our results illustrated the roles and mechanisms of *Prdx 6* in CLP induced ALI. After CLP, deficiency of *Prdx 6* caused more severe protein leakage and neutrophils accumulation in alveolar space. The oxidative stress, inflammation and matrix degradation were intensified more severely in *Prdx 6* (−/−) mice as compared to wildtype mice after CLP. Moreover, the activity of NF-κB was significantly increased after CLP and was much higher when *Prdx 6* was deficiency. These results demonstrated that deficiency of *Prdx 6* aggravated CLP-induced lung injury by enhancing the oxidative stress and inflammation.

ALI/ARDS is orchestrated by multiple inflammatory mediators and effector cells. The main underlying mechanisms are the uncontrolled inflammatory reactions and immunologic disturbances in presence of excessive cytokines and inflammatory mediators [16]. CLP could result in sepsis, which is a typical pathogenesis for ALI/ARDS. Accumulating evidence shows that sepsis is not only about the inflammation but also associated with imbalance in reactive oxygen species (ROS) production and consumption.

The roles of *Prdx*s in inflammation and oxidative stress have been noticed. *Prdx 6* has been reported to play important roles in scavenging ROS in hyperoxia and parquet induced lung injury [5,6], cigarette smoke-mediated lung inflammation [17], and hepatic ischemia-reperfusion induced liver injury in mice [18]. Moreover, consistent to our previously report of *Prdx 6* [7], *Prdx 1*, *Prdx 2* and *Prdx 3* were also demonstrated to present anti-inflammatory effects in LPS-induced injury [19–21]. But to date, the roles and mechanisms of *Prdx 6* in oxidative stress and inflammation are not fully understood.

MPO exists mainly in monocytes and neutrophils and could be released in abundance by neutrophils in the presence of extrinsic stimuli. MPO can reflect the level of neutrophils infiltration. In our study, the inflammation level of lung tissue after CLP was analyzed through pathologic changes and lung tissue MPO levels. Inflammatory cells infiltration was obviously observed after CLP, indicating typical pathologic changes of ALI and these pathologic changes were more severe in *Prdx 6* (−/−) mice. In addition, the MPO level in lung tissue increased

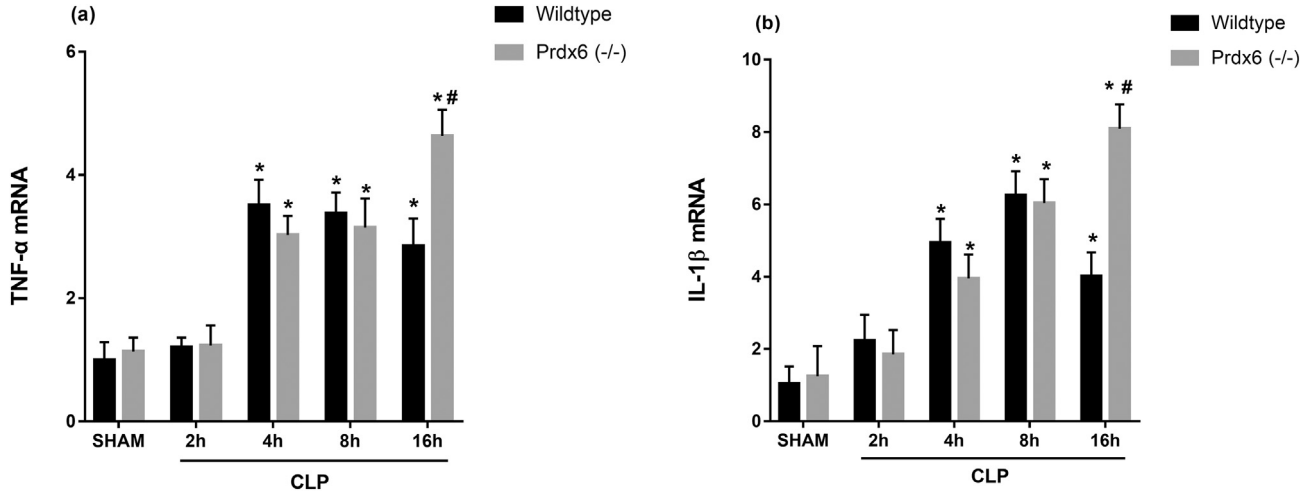

Fig. 5. The cytokines expression.
Peroxiredoxin (*Prdx*) *6*(−/−) and wild-type (WT) mice were subjected to cecal ligation and puncture (CLP) for 2 h, 4 h, 8 h, and 16 h. (A) The mRNA levels of tumor necrosis factor-α (TNF-α). The mRNA levels of TNF-α were significantly increased in *Prdx 6* (−/−) mice at 16 h after CLP and were markedly higher than those of the corresponding WT mice. (B) The mRNA levels of interleukin-1β (IL-1β). The mRNA levels of IL-1β were significantly increased in *Prdx 6* (−/−) mice at 16 h after CLP and were markedly higher than those of the corresponding WT mice. Values are expressed as means ± SD. *p < 0.05 as compared with vehicle-challenged mice; #p < 0.05 as compared with CLP-challenged WT mice (n = 6 in each group).

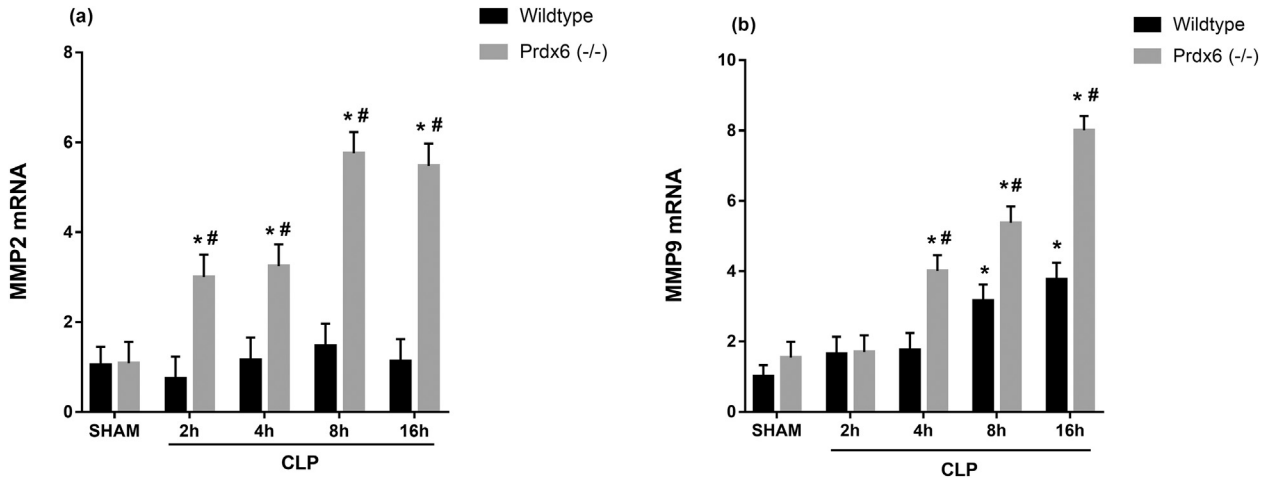

Fig. 6. The matrix metalloproteinases expression.
Peroxiredoxin (*Prdx*) *6*(−/−) and wild-type (WT) mice were subjected to cecal ligation and puncture (CLP) for 2 h, 4 h, 8 h, and 16 h. (A) The mRNA levels of matrix metalloproteinases (MMP) 2. The mRNA levels of MMP 2 were significantly increased in *Prdx 6* (−/−) mice from 2 h to 16 h after CLP and markedly higher compared to those of sham group and the corresponding WT mice. (B) The mRNA levels of MMP 9. The mRNA levels of MMP 9 were significantly increased in *Prdx 6* (−/−) mice from 4 h to 16 h after CLP and markedly higher compared to those of sham group and the corresponding WT mice. Values are expressed as means ± SD. *p < 0.05 as compared with vehicle-challenged mice; #p < 0.05 as compared with CLP-challenged WT mice (n = 6 in each group).

time-dependently, which was significantly higher in *Prdx 6* (−/−) mice than in C57BL/6J mice. These findings demonstrated that lung injury after CLP was aggravated when *Prdx 6* was deficiency.

SOD, CAT, and GPX are the main enzymes involving in oxidative stress [22]. SOD is the most potent enzyme to scavenge superoxide anions. MDA is the major product of lipid peroxidation and can reflect the level of ROS produced by lipid oxidation. Usually, MDA and SOD are analyzed simultaneously to evaluate the level of oxidative stress [23]. We demonstrated that oxidative stress was aggravated in a time dependent manner in lungs after CLP, which was indicated as higher levels of ROS (increased H_2O_2 and MDA), consumption of antioxidant enzymes (decreased total SOD activity, CuZn-SOD, GSH-PX, and CAT) and the T-AOC. These results are partially consistent with our previous reports in lung injury models induced by in situ lung ischemia/reperfusion and lung transplantation in rats [15] and by LPS instillation in mice [7]. All these oxidative stress parameters differed significantly between C57BL/6J mice and *Prdx 6* (−/−) mice at various time points, indicating that deletion of *Prdx 6* gene influenced the oxidative stress status under CLP. The results are understandable as *Prdx 6* is a peroxidase. After the gene was deleted, the protective effects against oxidative stress injury were then reduced.

Besides directly damaging the cells, ROS is involved in inflammatory reaction through upregulating the expression of NF-κB and multiple proinflammatory factors. NF-κB contributes to the transcriptional regulation of genes accounting for inflammation, immunologic reaction, cell proliferation and transformation [24,25]. Our results demonstrated NF-κB activities was elevated after CLP, which was more significantly higher in *Prdx 6* (−/−) mice as compared to that in C57BL/6J mice. Inflammation cascade is the typical process in ALI and is characterized by enhanced activity of nuclear factors and higher production of proinflammatory cytokines. We then tested the mRNA expressions of TNF-α and IL-1β and showed that they were upregulated time-dependently in the lung tissues of *Prdx 6* (−/−) mice and C57BL/6J mice when compared to the sham operated group.

MMPs could be produced by many matrix cells and inflammatory cells. MMP promoters include such domains as ETS, NF-κB and AP-1 [26,27]. MMP9 activity regulation is also closely associated with the expression of NF-κB related cytokines such as TNF-α and IL-1β [27,28]. In our study, infiltration of inflammatory cells in the lung alveolar space and increased level of MPO suggested that inflammatory cells were significantly increased after CLP. Then, they might secret more MMPs. At the same time, increased activity of NF-κB and elevated levels of cytokines as TNF-α and IL-1β could augment the production of MMPs. We then test the mRNA levels of MMP2 and MMP9. Interestingly, they were significantly increased after CLP and were more severe in *Prdx 6* (−/−) mice as compared to that in wildtype mice. MMP2 and MMP9 mainly decompose type IV collagen, a major component of basement membrane, which result in higher permeability of pulmonary alveolar capillary membrane [29]. We demonstrated permeability of pulmonary alveolar capillary membrane was significantly elevated after CLP through higher W/D ratio and the protein concentration in BALF. We further reported that those damage were significantly severe in *Prdx 6* (−/−) mice as compared to wildtype mice, which indicated the pivotal roles of *Prdx 6*.

In summary, *Prdx 6* gene deficiency aggravated CLP induced acute lung injury in mice via deletion of its antioxidative role. The mechanisms may be related to the followings: increased ROS; ROS-associated upregulation in the expression of NF-κB and its mediated cytokines like TNF-α and IL-1β, which resulted in a cascade of inflammatory reaction; NF-κB-related modulation of MMP2 and MMP9 expression, which directly led to degradation of extracellular matrix and resulted in increased pulmonary edema.

Abbreviations

ALI	acute lung injury
ARDS	acute respiratory distress syndrome
ROS	reactive oxygen species
Prdx	peroxiredoxins
CLP	cecal ligation and puncture
W/D	wet/dry ratio
BALF	bronchoalveolar lavage fluid
MPO	myeloperoxidase
H_2O_2	hydrogen peroxide
MDA	malondialdehyde
SOD	superoxide dismutase
XOD	xanthine oxidase
GSH-PX	glutathione peroxidase
CAT	catalase
TAOC	total anti-oxidative capability
NF-κB	nuclear factor-κB
cDNA	complementary deoxyribonucleic acid

TNF-α	tumor necrosis factor-α
IL-1β	interleukin-1β
MMP	matrix metalloproteinases

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Conflicts of interest

The authors have not disclosed any potential conflicts of interest.

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