PTPRO knockdown protects against inflammation in hemorrhage shock-induced acute lung injury involving the NF-κB signaling pathway

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Research Article

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Abstract

Background

Hemorrhage shock (HS) is characterized by decreased tissue oxygenation and organ damage due to severe blood loss. Acute lung injury (ALI) is a common complication of HS. Protein tyrosine phosphatase receptor type O (PTPRO) is abnormally up-regulated in the rat lungs after trauma/HS.

Methods

To elucidate the regulatory mechanism of PTPRO in lung inflammation following HS, we established a rat model of HS via withdrawing blood by a catheter inserted into the femoral artery followed by resuscitation. The rats were infected with lentiviral encoding PTPRO short hairpin RNA (shRNA) by intratracheal instillation for PTPRO knockdown.

Results

PTPRO was significantly up-regulated in rat lungs after HS. PTPRO knockdown enhanced epithelial integrity and reduced capillary leakage by up-regulating tight junction proteins zonula occludens-1 (ZO-1) and occludin (OCC). Besides, decreased myeloperoxidase expression and activity in the lungs indicated that HS-induced neutrophil infiltration into the lungs was mitigated by PTPRO knockdown. Meanwhile, expression of inflammatory cytokines/chemokines TNF-α, IL-6, MIP-2, MCP-2, and KC in the lungs or bronchoalveolar lavage fluid was regressed after PTPRO knockdown. The nuclear factor kappa B (NF-κB) pathway was related to inflammation in organ injury. PTPRO down-regulation had an inhibitory effect on the NF-κB pathway activation by suppressing the phosphorylation of NF-κB and its translocation from the cytoplasm into the nucleus in HS.

Conclusion

Taken together, we demonstrated that PTPRO knockdown may contribute to attenuating inflammation in HS-induced acute lung injury via inhibiting NF-κB pathway activation.

Introduction

Hemorrhagic shock (HS), a critical disease with a high mortality rate, occurs in patients with uncontrolled bleeding including trauma, maternal hemorrhage, and gastrointestinal hemorrhage, resulting in a circulatory dysfunction leading to decreased tissue oxygenation, organ damage, and even death [1–4]. About 1.9 million deaths result from HS per year worldwide [5]. HS initiates the inflammatory responses which facilitate organ injury and early multiple organ failure [6]. Acute respiratory distress syndrome (ARDS), a serious result of acute lung injury (ALI), is an important organ dysfunction syndrome caused by

trauma/HS [7]. HS contributes to the development of ALI through exacerbating inflammation and subsequent immunosuppression in the lungs, which results in nosocomial infections and secondary complications, thus leading to the final lung dysfunction and mortality [4].

Protein tyrosine phosphatase receptor type O (PTPRO) is an integral membrane protein belonging to the phosphor tyrosine phosphatases (PTPs) family and regulating tyrosine phosphorylation in cells [8]. In adult tissues, PPTPRO is widely expressed in the lung, heart, brain, and other organs [9]. According to the previous study, PTPRO aggravates atherosclerosis by promoting oxidative stress and cell apoptosis induced by oxidized low-density lipoprotein [10]. Oestrogen restricts renal podocyte apoptosis by inhibiting PTPRO [11]. Besides, PTPRO is a candidate tumor suppressor in human lung cancer and down-regulation of PTPRO mediated by miR-6803-5p promotes the proliferation and invasion of the cancer cells in colorectal cancer [12, 13]. Recently, more and more researches focus on the role of PTPRO in inflammation. For instance, PTPRO has an inflammation promotion effect on fulminant hepatitis and ulcerative colitis [14, 15]. PTPRO is up-regulated in placental mononuclear cells in patients with preeclampsia and its down-regulation mediated by miR-548c-5p overexpression plays an anti-inflammatory role in preeclampsia [16]. In the lungs, the role of PTPRO has only been reported in human lung cancer. According to the information retrieved from the Gene Expression Omnibus (GEO) DataSets (https://www.ncbi.nlm.nih.gov/gds), PTPRO is abnormally up-regulated in the rat lungs at 3 h post-trauma/HS (GSE6332). However, the functions of PTPRO in lung inflammation following HS are poorly elucidated.

The nuclear factor kappa B (NF-κB) pathway is considered a pro-inflammatory signaling pathway [17]. This pathway is strongly involved in the systemic inflammatory changes and immune response following hemorrhage shock [18]. Ethyl pyruvate restrains systemic leukocyte activation via regulating NF-κB after HS [19]. Inhibition of the activation of NF-κB reduces circulatory failure and organ injury and dysfunction in HS [20]. According to these findings, the reduction of the NF-κB pathway activation contributes to attenuating HS-induced inflammation and organ injury.

A series of researches have reported that PTPRO aggravates inflammation by activating the NF-κB signaling pathway [10, 14, 15]. There is little information available in the literature about the role of PTPRO in HS-induced inflammation and lung injury. Based on the previous results, we hypothesized that PTPRO functions in ALI following HS via regulating the NF-κB signaling pathway. We conducted this study to explore the expression and potential regulatory mechanism of PTPRO in lung inflammation following HS, which may contribute to the understanding of ALI pathogenesis following HS and indicate PTPRO as a potential therapeutic target for attenuating lung dysfunction induced HS.

Materials And Methods

Animal models

Twelve-week-old Sprague-Dawley rats purchased from Liaoning Changsheng Biotech Biotech Co., Ltd. (Benxi, China) were used for the HS model. The rats were allowed free access to food and water under a

condition of 22 ± 1°C and 12-h light/dark cycle for a one-week acclimation period. The femoral artery was cannulated with a catheter for withdrawing blood until mean arterial pressure (MAP) was decreased to 40–50 mmHg. MAP was maintained at this level for 60 min. After 1 h of shock, the rats were resuscitated with Lactated Ringer's solution for blood pressure restoration. Three hours after resuscitation, all rats were euthanized for tissue collection. The sham-operated rats underwent the same catheter implantation procedure but without bloodletting or resuscitation. The body temperature of the rats was maintained at 37°C during the surgery. For knocking down PTPRO in the rats, 48 h before hemorrhage by a catheter inserted into the femoral artery, the rat was infected with 1×10^8 TU lentiviral encoding PTPRO short hairpin RNA (shRNA) or its negative control shRNA (shNC) by intratracheal instillation through the mouth (Figure 1b). The rats were categorized into four groups (n=6 per group). This study was performed following the Institution Animal Care and Use Committee of Wuxi 9th Affiliated Hospital of Soochow University. After euthanasia, the blood, bronchoalveolar lavage fluid (BALF), and lung tissues were collected and stored at -70°C or fixed with 4% paraformaldehyde.

Clinical study

A clinical study was conducted in 7 patients with severe HS after polytrauma from Wuxi 9th Affiliated Hospital of Soochow University. Thirty healthy participants served as the controls. The blood samples were collected from all subjects after obtaining written informed consent. The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Wuxi 9th Affiliated Hospital of Soochow University (KT2020029).

Capillary leakage

Evans blue (EB) dye extravasation was performed to assess capillary leakage in the lungs. Twenty minutes before euthanasia, the rats were subjected to the tail vein injection with EB dye (50 ml/kg; Aladdin, Shanghai, China). After being euthanized, the heart was perfused with saline to remove the redundant dye in the vessels. EB was extracted from the lung tissues by incubation in formamide (4 m/g tissue; Aladdin, Shanghai, China) at 60°C for 48 h. The supernatant was detected with a microplate reader (800TS; BioTek, Winooski, VT, USA) at 620 nm after centrifugation. EB content was calculated according to a standard curve of EB in formamide and used to represent extravasation.

Quantitative real-time PCR

Using the Total RNA Isolation Kit (Tiangen Biotech Co. Ltd., Beijing, China) and BeyoRT II M-MLV reverse transcriptase (Beyotime, Shanghai, China), total RNA was isolated from the lungs and reverse transcribed into the first cDNA. A quantitative PCR assay was carried out by Exicycler^{TM} 96 Real-time PCR System (Bioneer Corporation, Daejeon, Korea) using SYBR Green (Solarbio, Beijing, China). The level of mRNA was normalized to GAPDH. The primers were synthesized by Genscript Biotechnology Co., Ltd. (Nanjing, China), and the sequences were shown in Table 1.

Western blot analysis

The lung tissues separated from the rats were lysed using RIPA buffer (Solarbio, Beijing, China) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF; Solarbio, Beijing, China) to isolate total proteins. Nuclear proteins were extracted using Nuclear Protein Extraction Kit (Solarbio, Beijing, China) according to the manufacturer's instructions. Protein quantified by bicinchoninic acid (BCA) Kit (Solarbio, Beijing, China) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, proteins were transferred onto a polyvinylidene difluoride membrane (PVDF; Millipore, Billerica, MA, USA). The membrane was blocked with 5% skim milk (Sangon Biotech, Shanghai, China) for 1 h. Afterward, the membrane was incubated with the primary antibody including PTPRO antibody (1:500 dilution; Affinity, Cincinnati, OH, USA), p-IKKα/β antibody (1:2000 dilution; Affinity, Cincinnati, OH, USA), IKKα/β antibody (1:1000 dilution; Affinity, Cincinnati, OH, USA), p-P65 (S536) antibody (1:1000 dilution; ABclonal, Shanghai, China), NF-κB (P65) antibody (1:500 dilution; ABclonal, Shanghai, China), GAPDH antibody (1:10000 dilution; Proteintech, Rosemont, IL, USA), and Histone H3 antibody (1:5000 dilution; GeneTex, Alton Pkwylrvine, CA, USA) overnight at 4°C, followed by incubation with the secondary anti-rabbit or anti-mouse horseradish peroxidase-linked antibodies (1:300 dilution) at 37°C for 1 h. Protein bands were visualized using electrochemiluminescence (ECL) reagent (Solarbio, Beijing, China). Band intensities were analyzed with Gel-Pro-Analyzer software and normalized to band intensity of GAPDH or Histone H3.

Enzyme-linked immunosorbent assay (ELISA)

The levels of tumor necrosis factor α (TNF-α) and interleukin (IL)-6 in BALF were detected with respective specific ELISA kits (LIANKE Biotech, Hangzhou, China) according to the manufacturer's instructions.

Histologic Analysis

Histological changes in the lungs were detected with hematoxylin and eosin (H&E) staining. The lung tissues fixed with 4% paraformaldehyde were embedded in paraffin and cut into 5-μm sections. After dewaxing and rehydration, the slides were stained with hematoxylin (Solarbio, Beijing, China) and eosin (Sangon Biotech, Shanghai, China). The images were captured under a microscope (BX53, Olympus, Tokyo, Japan) at 200 × magnification.

Immunofluorescence staining

The lung tissue slides embedded in paraffin were exposed to citrate solution for antigen retrieval after dewaxing and rehydration. The slides were blocked with goat serum (Solarbio, Beijing, China) at room temperature for 15 min and incubated with MPO antibody (1:100 dilution; ABclonal, Shanghai, China) or P65 antibody (1:100 dilution; ABclonal, Shanghai, China) overnight at 4°C. Then the tissues were incubated in the dark with a Cy3-conjugated goat secondary antibody (1:200 dilution; Beyotime, Shanghai, China) at room temperature for 1 h. The tissues were re-stained with 4',6-diamidino-2-phenylindole (DAPI; Beyotime, Shanghai, China), and the images were captured by a fluorescence microscope (BX53, Olympus, Tokyo, Japan).

Myeloperoxidase (MPO) activity

Infiltration of neutrophils into the lung tissue was assessed by MPO activity. Myeloperoxidase assay kit (Jiancheng Bioengineering Institute, Nanjing, China) was used for the detection of MPO activity in the lungs according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using GraphPad Prism 7.0 and expressed as mean ± standard deviations (SD). The values of different groups were compared with one-way analysis of variance (ANOVA) followed by Tukey's test. Significance was considered when $P < 0.05$ between different groups.

Results

HS strongly induces the expression of PTPRO in rat lungs.

The serum samples were collected from 7 HS patients and 30 healthy participants. PTPRO was shown to be highly expressed in the serum of HS patients (Fig. 1a). Dysregulation of PTPRO in HS patients suggests a potential role of PTPRO in HS pathogenesis, we further investigated PTPRO expression in the lung of rats. As shown in Fig. 1c and 1d, HS rats exhibited higher PTPRO expression in the lungs both at mRNA and protein level compared to sham-operated rats. When infected with lentiviral harboring PTPRO shRNA, HS rats displayed a significantly decreased PTPRO expression level. These indicated that HS strongly induced PTPRO expression in rat lungs and infection with lentiviral successfully knocked down this gene in the rats.

PTPRO knockdown reduces capillary leakage and attenuates HS-induced lung injury.

The severity of the lung injury induced by HS was detected with H&E staining. As the results showed, alterations in the alveolar-capillary barrier, more compact lung parenchyma, alveolar septal thickening, and inflammatory cell infiltration occurred in the lungs due to HS, which were significantly attenuated by LV-shPTPRO (Fig. 2a). The content of EB dye in the lung tissues was detected for lung permeability assessment. EB content in the lungs of HS rats was dramatically increased compared to those of sham-operated rats and it was significantly reduced when PTPRO expression was repressed (Fig. 2b). Moreover, expression of tight junction proteins zonula occludens-1 (ZO-1) and occludin (OCC), biomarkers of epithelial integrity, was markedly decreased in the lung by HS, while it was partially restored after PTPRO knockdown (Fig. 2c). These findings suggest that down-regulation of PTPRO enhances the epithelial integrity and reduces HS-induced capillary leakage through up-regulating ZO-1 and OCC in the lungs.

Down-regulation of PTPRO attenuates neutrophil infiltration in the lungs.

Immunofluorescence staining and activity measurement of MPO showed that the expression and activity of MPO were enhanced in the lungs by HS. PTPRO knockdown markedly reduced post-shock MPO expression and activity, indicating that HS-induced neutrophil infiltration into the lungs was mitigated by

PTPRO expression suppression (Fig. 3a and b). Therefore, down-regulation of PTPRO could alleviate HS-induced neutrophil infiltration in rat lungs.

PTPRO down-regulation decreases the lung levels of inflammatory cytokines/chemokines in HS.

The expression of inflammatory cytokine or chemokine including TNF-α, IL-6, macrophage inflammatory protein (MIP)-2, monocyte chemoattractant protein (MCP)-2, and keratinocyte chemoattractant (KC) in the lung tissues was analyzed. As shown in Fig. 4a, the mRNA levels of pro-inflammatory cytokines TNF-α and IL-6 in the lungs of HS rats were significantly up-regulated compared to those of sham-operated rats, while they were regressed due to suppression of PTPRO expression. Also, the levels of TNF-α and IL-6 in BALF detected with ELISA exhibited similar alterations as their mRNA expression (Fig. 4b). Furthermore, HS increased expression of inflammatory chemokines MCP-2, a chemoattractant to macrophages, and KC and MIP-2, chemoattractants to neutrophils, in the lungs, while expression of the genes in HS rat lungs was reversed by PTPRO down-regulation (Fig. 4c), indicating that inflammation in HS rat lungs was mitigated when PTPRO was inhibited.

Knockdown of PTPRO contributes to the suppression of the NF-κB signaling pathway activation in HS.

The expression of the proteins involved in the NF-κB signaling pathway was analyzed. Immunofluorescence staining revealed that NF-κB P65 was mainly expressed in the cytoplasm and HS promoted the translocation of NF-κB P65 from the cytoplasm into the nucleus. Down-regulation of PTPRO abolished the effect of HS on NF-κB P65 translocation (Fig. 5a). Western blot analysis showed that HS enhanced the p-IKKα/β level without altering total IKKα/β expression. The ratio of p-IKKα/β protein level to total IKKα/β level increased by HS dramatically declined when PTPRO was knocked down, which demonstrated that HS promoted IKKα/β phosphorylation while PTPRO knockdown restricted this process in the lungs (Fig. 5b). The level of phosphorylated NF-κB P65 increased by HS was also regressed by PTPRO down-regulation (Fig. 5c). Moreover, knockdown of PTPRO caused a decreased level of nuclear NF-κB P65 expression in the lungs of HS rats (Fig. 5d). PTPRO knockdown reduced the phosphorylation of IKKα/β and NF-κB P65 and inhibited the translocation of NF-κB P65 from the cytoplasm into the nucleus, thus restricting the activation of the NF-κB signaling pathway.

Discussion

In this study, our results confirm that knockdown of PTPRO mitigates acute lung injury after HS by reducing inflammation, which may be associated with the suppression of the NF-κB signaling pathway.

PTPRO expression in the lungs after HS is not explored. In the current study, we found that PTPRO was significantly up-regulated in the lung of HS rats. Similarly, expression of PTPRO truncated isoform (PTPROt) is increased in liver macrophages with the increasing degree of nonalcoholic steatohepatitis [21]. In contrast, PTPRO is down-regulated in injured podocytes and lung squamous cell carcinoma compared with healthy tissues [22, 23]. PTPRO may exhibit different expression alterations in different organs or diseases. We found that in HS-induced lung injury, histologic architecture damages in the lungs

were alleviated and capillary permeability was reduced by PTPRO knockdown, with up-regulation of ZO-1 and OCC, two markers of epithelial integrity. Up-regulation of PTPRO and the functions of PTPRO knockdown in lung injury indicate that PTPRO is likely to contribute to the progression of HS-induced lung injury and capillary leakage.

Inflammation is a major cause of the development of ALI following HS. In histologic analysis, down-regulation of PTPRO reduced HS-induced inflammatory cell infiltration into the lungs. Besides, as a marker for neutrophil activation, MPO expression and activity in the lungs were reduced by PTPRO down-regulation, which suggested that there were fewer neutrophils infiltrated into the lungs. Expression of inflammatory cytokine/chemokine such as TNF-α, IL-6, MCP-2, KC, and MIP-2 in the lungs is reported to be up-regulated after HS [24]. Consistent with this finding, our results showed that the expression levels of these inflammatory cytokines /chemokines were increased in the lungs after HS. In our study, they were reduced when PTPRO expression was inhibited. KC and MIP-2 are neutrophil infiltration chemokine markers and MCP-2 is a specific chemokine marker of macrophage infiltration. The results in this part reveal that PTPRO knockdown suppresses inflammatory cell infiltration into the lungs and plays an anti-inflammatory role in HS-induced ALI. The role of PTPRO in the inflammatory response in the lungs remains unexplored. However, the previous result that miRNA-548c-5p, a microRNA targeting PTPRO, suppresses inflammatory response in preeclampsia by down-regulating PTPRO also suggests that PTPRO may facilitate inflammation in preeclampsia, which is similar to our findings [16].

Inflammation in lung injury is strongly associated with the NF-κB signaling pathway. miRNA-1246 increases NF-κB protein expression and induced inflammation in the ALI cell model, while NF-κB inhibitor suppresses miRNA-1246-induced inflammation [25]. Inhibition of p38MAPK/NF-κB pathway reduces the inflammatory response in lipopolysaccharide-induced acute lung injury [26]. In trauma/HS-induced lung injury, ethyl pyruvate reduced inflammation and cell apoptosis by inhibiting the NF-κB pathway [27]. Therefore, these findings indicate that suppression of the NF-κB pathway plays an anti-inflammatory role in HS-induced lung injury. Recently, PTPRO has been reported to exaggerate inflammation in liver macrophages and ulcerative colitis through enhancing NF-κB activation [15, 28]. Based on these findings, we wondered if PTPRO regulated inflammation in HS via the NF-κB signaling pathway. Therefore, in our study, the expression and phosphorylation of components in the canonical NF-κB signaling pathway were analyzed. As predicted, IKKα/β phosphorylation in the lungs was reduced by PTPRO knockdown in HS. It is IKKβ, not IKKα that is required for activation of the canonical NF-κB signaling pathway through activation of IκBs [29]. This finding suggests that PTPRO knockdown reduced IKKβ phosphorylation in HS. Likewise, PTPRO down-regulation also restrained the NF-κB P65 phosphorylation and translocation of NF-κB P65 from the cytoplasm into the nucleus. These results indicate that PTPRO knockdown reduces the activation of the NF-κB signaling pathway in HS-induced lung inflammation, which is similar to a previous finding that miR-548C-5p reduces the inflammatory response in preeclampsia by targeting PTPRO and inhibiting the NF-κB pathway [16]. Therefore, PTPRO knockdown may suppress inflammation in HS-induced lung injury via inhibiting the NF-κB signaling pathway.

NF-κB lies downstream of antigen receptors, growth factors, and cytokine receptors including TNF receptor family. A diverse range of stimuli including LPS, cytokines TNF-α and IL-1, reactive oxygen species, and other factors can activate this dimer [30]. NF-κB P65 comprises the predominant NF-κB transcriptional activity and it is responsible for the expression of a large number of proinflammatory mediators [31]. In the current research, HS induced the expression of PTPRO in the lungs, which may activate the NF-κB phosphorylation and its translocation from the cytoplasm into the nucleus, thus initiating the transcription of pro-inflammatory genes including cytokines TNF-α and IL-6, and chemokines MIP-2, MCP-2, and KC in the lungs. The recruitment and infiltration of macrophages and neutrophils triggered inflammation in the lungs (Fig. 6). Inflammation and disruption of tight junctions between alveolar epithelial cells eventually lead to the breakdown of the epithelial barrier and enhanced lung permeability [32]. However, PTPRO knockdown attenuates HS-induced inflammation and lung injury as well as reduces the NF-κB pathway activation in HS, which suggests that PTPRO knockdown is likely to protect against HS-induced inflammation through inhibiting the NF-κB signaling pathway in the lungs. Nevertheless, in the present study, there was no more experiment able to confirm that PTPRO was involved in HS-induced lung inflammation via regulating the NF-κB signaling pathway. To make this finding more convincing, further exploration should be carried out in the future. For instance, the effects of knockdown of NF-κB P65 or other components in the NF-κB pathway on lung inflammation following HS could be investigated.

In conclusion, we demonstrate that PTPRO knockdown plays an anti-inflammatory role in HS-induced lung injury, which is associated with its inhibitory effect on NF-κB pathway activation. This finding indicates that PTPRO may be a potential therapeutic target for ameliorating lung injury in patients suffering from HS.

Declarations

Ethics approval and consent to participate

The animal experiments were approved by the Ethics Committee of Wuxi 9th Affiliated Hospital of Soochow University.

Consent for publication

Not Applicable.

Availability of data and materials

The data sets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Competing interests

The authors have declared that no competing interest exists.

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Author contributions

XG designed the study. ZRH, CX, YT and JMC performed the experiments and analyzed the data. CX and HY prepared the manuscript. FYB and YW composed the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. The primers used for RT-qPCR.

Gene	Forward	Reverse
PTPRO	TGCTCGGGCTCTTTGTGC	ATCGGGATGGTTTGGTGA
TNF-α	CGGAAAGCATGATCCGAGAT	AGACAGAAGAGCGTGGTGGC
IL-6	AACTCCATCTGCCCTTCA	CTGTTGTGGGTGGTATCCTC
KC	ACCCAAACCGAAGTCATAGC	GGGACACCCTTTAGCATCTT
MCP-2	ACAGCAATGCCCAGTTTA	ACCTCCAGACACCTTTCG
MIP-2	ACTGGTCCTGCTCCTCCT	TTAGCCTTGCCTTTGTTC
OCC	CAGAGCCTATGGAACGG	CAAGGAAGCGATGAAGC
ZO-1	ATCTCCAGTCCCTTACCTTTC	TGGTGCTCCTAAACAATCAG
GAPDH	CGGCAAGTTCAACGGCACAG	CGCCAGTAGACTCCACGACAT

Figures

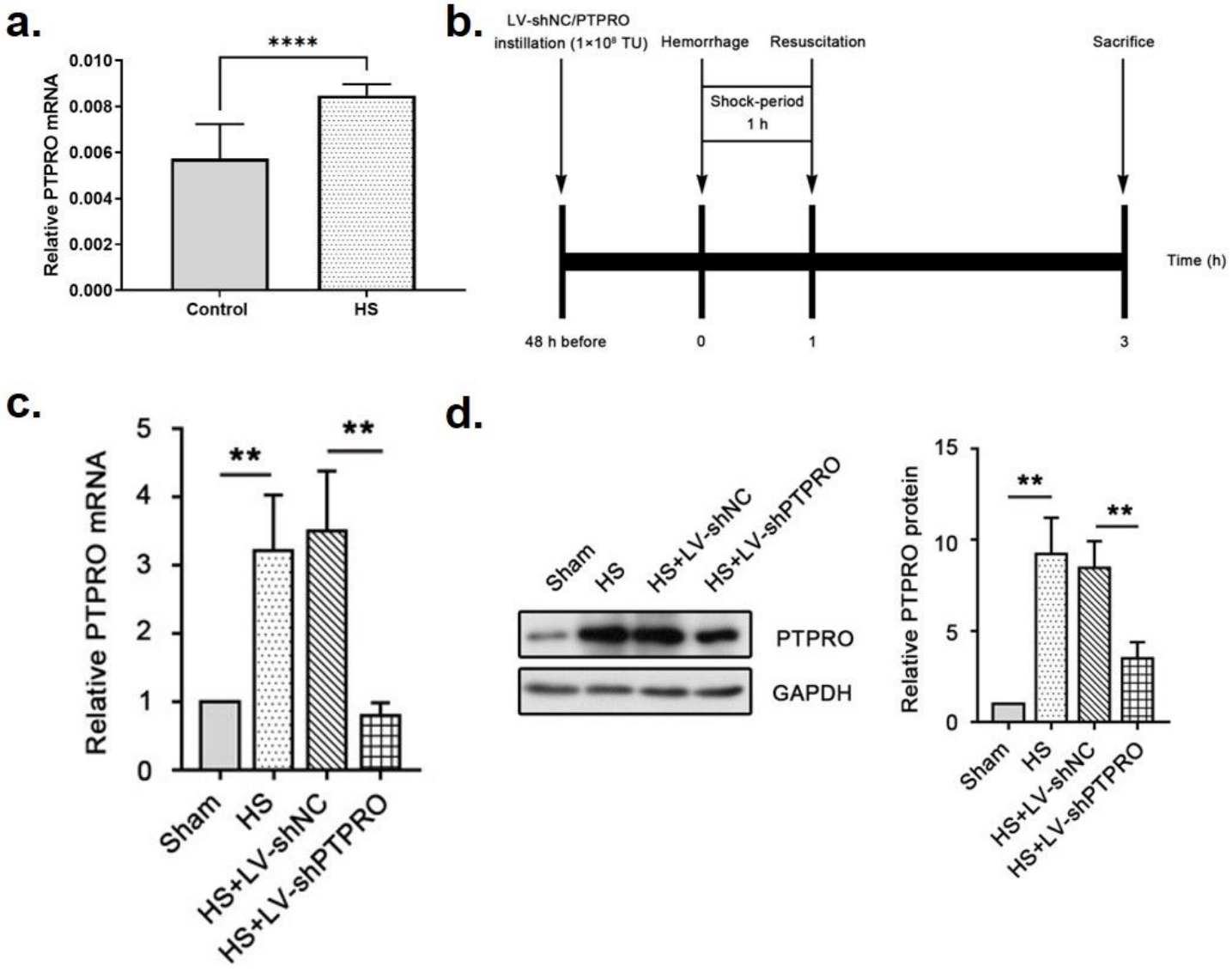

Figure 1

a. Increased expression of PTPRO in the serum of HS patients. RT-qPCR analysis of the expression of PTPRO in the serum of HS patients (n=7) versus healthy participants (n=30). **b.** Timeline of the experiments. Rats were subjected to withdrawing blood by a catheter inserted into the femoral artery. Mean arterial pressure was maintained at 40–50 mmHg for 60 min (shock-period) and then the rats were resuscitated. Three hours after resuscitation, all rats were euthanized for tissue collection. For PTPRO knockdown, the rats were instilled with lentiviral encoding PTPRO short hairpin RNA (shRNA) (LV-shPTPRO) or negative control shRNA (LV-shNC) 48 h before hemorrhage. **c-d.** HS strongly induces the expression of PTPRO in rat lungs. **c.** The relative mRNA expression of PTPRO in the lung of HS rats was detected by RT-qPCR. **d.** Representative image and relative PTPRO protein expression in rat lungs were detected by Western blot. The values represent the mean (n=6) ± SD. **P<0.01, ****P<0.0001.

Figure 2

PTPRO knockdown reduces capillary leakage and attenuates HS-induced lung injury. **a.** Lung histopathology in the HS model was assessed by H&E staining. Magnification: 200 ×. Scale = 100 μm. **b.** Micrograms of Evans blue per gram of lung tissue were used to assess lung permeability in HS model. **c.** Relative mRNA levels of tight junction proteins zonula occludens-1 (ZO-1) and occludin (OCC) in the lung tissues were determined by RT-qPCR. Values are means (n=6) ± SD.**P<0.01.

Figure 3

Down-regulation of PTPRO attenuates neutrophil infiltration in the lungs. **a.** Representative images of myeloperoxidase (MPO) immunofluorescence staining in the lung of HS rats were shown. Magnification: 400 ×. Scale = 50 μm. **b.** MPO activity in the lung tissues of HS rats was detected with the MPO kit. Error bars show SD. N=6, **P<0.01.

Figure 4

PTPRO down-regulation decreases the lung tissue levels of inflammatory cytokines/chemokines in HS. **a.** Relative mRNA levels of pro-inflammatory cytokines TNF-α and IL-6 were detected by RT-qPCR. **b.** The levels of TNF-α and IL-6 in bronchoalveolar lavage fluid (BALF) were detected with ELISA. **c.** Relative mRNA levels of chemokines MCP-2, KC, and MIP-2 in the lung tissues. Bars indicate SD. N=6, **P<0.01.

Figure 5

Knockdown of PTPRO contributes to the suppression of the NF-κB pathway activation in HS. **a.** Immunofluorescence staining showed the intracellular localization of NF-κB P65 in the lung tissues. Magnification: 400 ×. Scale = 50 μm. **b.** Expression levels of total IKKα/β and phosphorylated IKKα/β in the rat lung were detected by Western blot and the ratio of p-IKKα/β to total IKKα/β was calculated. **c-d.** Western blot and expression quantification of phosphorylated NF-κB P65. **c.** and nuclear NF-κB P65. **d.** in the lungs were performed. Values are means (n=6) ± SD. **P<0.01.

Figure 6

Diagram of the potential regulatory mechanism of PTPRO in HS-induced lung inflammation. PTPRO is up-regulated in the lungs after HS, which may contribute to NF-κB P65 phosphorylation and the

translocation of NF-κB from the cytoplasm into the nucleus. Activated NF-κB in the nucleus likely initiates the transcription of proinflammatory genes including cytokine and chemokine, thus inducing lung inflammation following HS. PTPRO knockdown alleviates the HS-induced inflammation in the lungs by reducing the NF-κB pathway activation.