

SHP2 inhibitor PHS1 ameliorates acute kidney injury by Erk1/2-STAT3 signaling in a hemorrhage followed CLP mice model

Jihong Jiang

Shanghai General Hospital/Shanghai Jiao Tong University School of Medicine

Baoji Hu

Shanghai Pudong Hospital/Fudan University-Pudong Medical Center

Chun-Shiang Chung

Rhode Island Hospital/the Alpert School of Medicine at Brown University

Yaping Chen

Rhode Island Hospital/the Alpert School of Medicine at Brown University

Yunhe Zhang

Shanghai East Hospital/Tongji University

Jinbao Li

Shanghai General Hospital/Shanghai Jiao Tong School of Medicine

Alfred Ayala (✉ AAyala@lifespan.org)

Rhode Island Hospital/the Alpert School of Medicine at Brown University <https://orcid.org/0000-0002-5034-2995>

Research article

Keywords: Phosphatase inhibition, AKI, hemorrhage, shock, sepsis

Posted Date: April 17th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-21944/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published on September 21st, 2020. See the published version at <https://doi.org/10.1186/s10020-020-00210-1>.

Abstract

Background

Hypovolemic shock and septic challenge are two major causes of acute kidney injury (AKI) in the clinic course. Src homology 2 domain-containing phosphatase 2 (SHP2) is one of the major phosphatase protein tyrosine phosphatase (PTPs), which play a significant role in maintaining immunological homeostasis by regulating many facets of immune cell signaling. In this study, we explored whether SHP2 signaling contributed to AKI induced by hemorrhage (Hem) followed by cecal ligation and puncture (CLP) (Hem/CLP) and, further, if inactivation of SHP2 with the selective inhibitor, Phenylhydrazonopyrazolone sulfonate 1 (PHPS1), attenuated this injury.

Methods

Male C57BL/6 mice were subjected to Hem (a “priming” insult) followed by CLP or sham-Hem plus sham-CLP (S/S) as controls. Samples of blood and kidney were harvested at 24 h post CLP. The expression of neutrophil gelatinase-associated lipocalin (NGAL), high mobility group box 1 (HMGB1), caspase3 as well as SHP2:phospho-SHP2, extracellular-regulated kinase (Erk1/2):phospho-Erk1/2, and signal transducer and activator of transcription 3 (STAT3):phospho-STAT3 protein in kidney tissues were detected by Western blotting. The levels of creatinine (Cre) and blood urea nitrogen (BUN) in serum were measured according to the manufacturer’s instructions. Blood inflammatory cytokine/chemokine levels were detected by ELISA.

Results

Here we report that indices of kidney injury, such as BUN, Cre, NGAL in renal tissue and histopathologic change, were pronounced after Hem/CLP in comparison with that observed in the S/S group mice. Furthermore, while Hem/CLP elevated serum levels of inflammatory cytokine/chemokine, and induced increased levels of HMGB1, SHP2:phospho-SHP2, Erk1/2:phospho-Erk1/2, as well as STAT3:phospho-STAT3 protein expression in the kidney; treatment with PHPS1 markedly attenuated these Hem/CLP induced changes.

Conclusions

In conclusion, our data indicate that SHP2 inhibition attenuates AKI induced by our double-hit/sequential insult model of Hem/CLP and that this protective action may be attributable to its anti-inflammatory property in mitigating activation of the Erk1/2 and STAT3 signaling pathway. This finding we believe has important potential clinical implications and, thus, warrants further investigation.

Introduction

Trauma patients often suffer with hypovolemic shock and septic challenge simultaneously, which results in severe organ dysfunction (Karasu et al. 2019, Spahn et al. 2019). The kidneys, are one of the most commonly affected organs, receiving 20–25% of the resting cardiac output, while their weight is less than 1% of the total body mass. Hypothetically, making them more vulnerable and susceptible to the effect of blood volume loss and fluid shifts associate with sepsis, resulting in acute kidney injury (AKI). And while significant aspects of the pathogenesis of AKI have lead to better diagnostics/identification of clinical risk factors, so that supportive therapies are provided more judiciously (Honore et al. 2015); the mortality rates of AKI remain unacceptably high (Barbar et al. 2018, Uchino et al. 2005, White et al. 2013). Inasmuch; it is important to focus on the pathological mechanism that allow us to identify novel therapeutic targets so that we might leverage them to better treat the pathological development of AKI in the critically ill/severely injured patient.

The pathophysiology of AKI is thought to be induced by ischemic and/or septic events that lead to metabolic derrangments (Dellepiane et al. 2016). These derrangments are thought to be, in part, a result of the dys-balance between the system inflammatory response syndrome (SIRS) and/or anti-inflammatory response that develops in the shocked/ septic patient/ experimental animal (Poston and Koyner 2019). Exposure to pro-inflammatory cytokines are thought to lead to renal cell death and dysfunction (Gomez et al. 2014). The administration of antibiotics directed at curbing septic pathogens also has been shown not to reduce the mortality rate associated with the development of AKI during the clinic course of the critically ill patient (Kellum et al. 2019). We and others have revealed that immune co-inhibitory receptor molecules appear to be involved in the pathology of the shocked/septic experimental animal's inflammatory response (Patil et al. 2017, Biron et al. 2018). As co-inhibitory receptors of the programmed cell death receptor-1 (PD-1) family bear an intracellular tyrosine inhibitory motif (ITIM) and/or intracellular tyrosine switch motif (ITSM), it has been shown that the suppression of T-cell function is a result of the recruitment of the phosphatase protein tyrosine phosphatase (PTP) family members (Keir et al. 2008, Riley 2009).

Src homology 2 domain-containing phosphatase 2 (SHP2) is one of the major PTPs. As such; it plays a significant role in maintaining immunological homeostasis by regulating many facets of immune cell signaling. Chichger et al. found that SHP2 activation protects the endothelial barrier against injury in the lung (Chichger et al. 2015). We recently reported that SHP1, but not SHP2, negatively modulated PD-L1 dependent regulation of T regulatory lymphocyte (Treg) function and in so doing contributed to resolving shock/sepsis-induced lung injury (Tang et al. 2015). At the same time, some studies have revealed that SHP2 deficiency may have a protective effect in experimental renal (Hsu et al. 2017, Teng et al. 2018, Verma et al. 2016) and cardiovascular (Chen et al. 2018) conditions. Thus, SHP2 activation may play distinct roles in different organs. Activation of SHP2 serves the role of a positive modulator of extracellular-regulated kinase (Erk) activity, which is downstream of the induction of a number of cytokines (Maroun et al. 2000). In addition to its role in growth factor receptor-bound protein 2 (Grb-2)–associated binder 1 (Gab1)-mediated Erk activation, SHP2 attenuates epidermal growth factor (EGF)-

dependent phosphatidylinositol (PI)3 kinase activation by dephosphorylating Gab1 at the p85 binding sites (Zhang et al. 2002). It is thought that SHP2 is involved in the aspects of the inflammatory response that depend on Erk signaling. Thus, our study sets out to examine the hypothesis that the development of AKI, as a result of the sequential insults of hemorrhagic shock followed by septic challenge, is mediated, in part, through dysregulated SHP2 activation acting in-turn on Erk1/2 and signal transducers and activators of transcription (STAT)3.

Materials And Methods

Animals and groups

All experiments were performed in accordance with National Institutes of Health guidelines and approved by the Animal Use Committee of Rhode Island Hospital (AWC# 5064-18). A total of 18 male C57BL/6 mice (10 to 12 weeks old) were included in the experiment. The animals were maintained in a 12/12-hour light/dark cycle at ambient temperature (23–25 °C) and provided with standard laboratory rodent chow and water *ad libitum*.

Mice were randomly divided into three groups of 6 animals each: 1) the control group: mice underwent sham hemorrhage (Hem) and sham septic surgical procedure (S/S); 2) the Hem/cecal ligation and puncture (CLP) sepsis + vehicle group; and 3) the Hem/CLP + PHPS1 (inhibitor of SHP2) group.

Experimental protocol

Hemorrhage and sepsis were elicited as described previously in our laboratory (Biron et al. 2018). In brief, bilateral femoral arteries of mice were catheterized under anesthesia. The mean blood pressure of arteries was continuously monitored through one catheter attached to a blood pressure analyzer (MicroMed, Louisville, Ky). When recovered from anesthesia, the mice were bled over a 5 to 10 min period to a mean blood pressure of 35 ± 5 mmHg and kept stable for 90 min. Then, mice were resuscitated by infusion with four times drawn blood volume of Ringer's solution. Mice in the S/S group were anesthetized and restrained in a supine position, and blood vessels were ligated, but no blood was drawn.

Twenty four hours after Hem, mice were submitted to sepsis, as elicited by the CLP technique as described previously (Biron, et al. 2018). Briefly, mice were anesthetized by isoflurane and a midline abdominal incision was performed. The cecum was mobilized and ligated at its middle portion below the ileocecal valve, punctured twice using a 22-gauge needle, and a small stool sample was squeezed out of the cecum to induce polymicrobial peritonitis. The abdominal wall was closed in 2 layers. Mice in the S/S group underwent the same procedure, including opening of the peritoneum and exposing the bowel, but without ligation or needle perforation of the cecum. After surgery, the mice were resuscitated by a subcutaneous injection of pre-warmed (37 °C) 0.6 mL normal saline.

The SHP2 selective inhibitor, Phenylhydrazonopyrazolone sulfonate 1 (PHPS1) (Cayman Chemical, Ann Arbor, MI), at 3 mg (dissolved in DMSO/PBS = 1:1 solvent and)/kg body weight was administered by a

subcutaneous injection once immediately after Hem and, once again, following the performance of CLP. Twenty four hours after CLP (48 hours after Hem), mice were killed, blood and kidneys were collected for analyses.

Determination of serum biochemical indicators

Blood urea nitrogen (BUN) and creatinine (Cre) concentrations were measured using corresponding kits (Abcam, Cambridge, MA). Blood inflammatory cytokine/chemokine levels were detected by commercial ELISA kit (BD Biosciences, San Jose, CA). All procedures were performed according to the manufacturer's instructions.

Calculation of kidney/body weight index (KI).

The left kidney was harvested, weighed, frozen rapidly in liquid nitrogen and stored at -80°C for subsequent analysis. The kidney/body weight index (KI) was calculated as: kidney wet weight (mg)/total body weight (g) $\times 100\%$. Under normal conditions, KI is relatively constant; when the kidneys suffering congestion, edema even hypertrophy, the ratio of kidney to body weight increased (Zhang et al. 2019).

Renal histology

The right kidney was fixed in 4% formaldehyde, embedded in paraffin and cut into 5 μm thick sections. Hematoxylin and eosin (HE)-stained sections were scored in a blinded, semiquantitative manner using a 0 to 4 scale (Wu et al. 2019). Tubular damage such as tubular necrosis, dilatation, apoptosis, and cast formation was scored as follows: 0 (none), 1 (1–10%), 2 (11–25%), 3 (26–45%), and 4 (46–75%).

Western blot analysis

Whole tissue kidney protein samples/ lysates (30 μg total protein in each lane) were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis using a 10% gel and then transblotted onto polyvinylidene fluoride membranes. The protein levels of neutrophil gelatinase-associated lipocalin (NGAL), HMGB1, SHP2, phospho-SHP2 (Tyr580), Erk1/2, phospho-Erk1/2, STAT3, phospho-STAT3 and caspase 3 were determined using specific antibodies (1:1000; Cell Signaling). GAPDH and Beta-actin (1:3000; Abcam, Cambridge, USA) were used as loading control, and the amount of protein in the blots was quantified using a Bio-Rad ChemiDoc Imaging System and Image Lab 6.0 software (Bio-Rad Laboratories, California, USA).

Statistical analysis

Values are expressed as the mean \pm standard deviation. The statistical analysis was performed with the SPSS version 24.0 statistical software package (IBM Inc., Armonk, NY, USA). Data were tested for normality and equality of variance. Comparisons among the three groups for each dependent variable were performed using an analysis of variance (ANOVA) with a post hoc Newman-Keuls multiple comparison test. The level of statistical significance was set at $P < 0.05$.

Results

Hem/CLP-induced SHP2 activation in the kidney is attenuated by PHPS1 treatment

PHPS1, a cell-permeable highly selective inhibitor for SHP2, has been shown to inhibit SHP-2-dependent cellular signaling and tumor cell colony formation (Hellmuth et al. 2008). In this study, subcutaneous injection of mice with PHPS1 immediately after Hem and CLP procedure significantly decreased the level of activated SHP2 detected when compared with vehicle-treated mice as shown in Fig. 1.

Treatment with PHPS1 reduces the extent of Hem/CLP-induced kidney injury (KI).

Here, we observed a marked increase in the KI of Hem/CLP mice. This is important since an increased KI reflects kidney damage such as edema, hypertrophy and organ congestion. The KI in Hem/CLP group mice increased compared with S/S group, however, PHPS1 treatment suppressed this increase as shown in Fig. 2. This implies that PHPS1 treatment directly or indirectly reduced the kidney edema and cell hypertrophy occurring here.

SHP2 inhibition provides a renal protective effect in Hem/CLP mice.

As expected, mice subjected to Hem/CLP procedure exhibited a significantly elevated BUN and Cre levels in serum when compared to that of S/S control mice. Here again, treatment with PHPS1 significantly improved renal function by reducing the levels of BUN and Cre (Fig. 3A and 3B).

It has been reported that NGAL is a promising biomarker for diagnosing acute kidney injury (Kellum et al.). Here we attempted to detect changes in the expression of NGAL in the kidney by Western Blot. As shown in Fig. 3C, NGAL was significantly upregulated in renal tissue of both Hem/CLP group mice compared with S/S mice. Interestingly, Hem/CLP group administered the SHP2 inhibitor PHPS1 exhibited reduced NGAL levels compare to vehicle-treated Hem/CLP mice. We also examined the change induced in cleaved (activated) caspase 3 in renal tissue by Western Blot. Hem/CLP increased the expression of cleaved caspase 3 compared to S/S mice, however, the injection of Hem/CLP with PHPS1 attenuated the rise in cleaved caspase 3 to levels seen in S/S mice, when compared to vehicle treated Hem/CLP mice (Fig. 3D).

The changes in kidney histopathologic induced by Hem/CLP are attenuated by PHPS1 treatment.

As shown by histology examination in Fig. 4, Hem/CLP caused an increase in pathological changes in the kidney; including tubular epithelial cell sloughing, edema, inflammatory cell infiltration, loss of brush border, tubular dilation, and tubular distortion, when compared with that of S/S mice. However, PHPS1 treatment markedly ameliorated these changes and preserved the renal architecture.

The Hem/CLP-induced increase in systemic blood inflammatory cytokine/ chemokine levels is suppressed by PHPS1 administration.

The levels of inflammatory factors assessed included IL-6, TNF- α , IL-10, as well as chemokines KC and MIP-2 in blood that were significantly increased in the Hem/CLP group mice when compared with S/S group. Alternatively, while PHPS1 injection dramatically reduced the levels of TNF- α , IL-10, and MIP-2. Interestingly, there was no significant reduction/change observed in IL-6 and KC levels following Hem/CLP (Fig. 5).

PHPS1 treatment suppresses the Hem/CLP-induced expression of HMGB1 in the kidney.

HMGB1, as a late lethal inflammatory mediator, is reported to play an important role in sepsis course (Vijayakumar et al. 2019, Wang et al. 2001). We observed a significant upregulation of HMGB1 in the renal tissue of Hem/CLP group mice that was also abrogated in the PHPS1 treated Hem/CLP group (Fig. 6).

PHPS1 inhibits the Hem/CLP-induced activation of the Erk1/2-STAT3 signaling pathway.

To further illuminate the nature of the protective effects of PHPS1 on the development of AKI here, we chose to examine the extent to which not only SHP2, but Erk1/2 and STAT3, were activated/phosphorylated in renal tissue. As shown in Fig. 7, compared to S/S mice, Hem/CLP induced marked activation of SHP2, Erk1/2 and STAT3, as shown by the significant elevation in the ratio of phosphorylated-SHP2:total-SHP2, phosphorylated-Erk1/2:total-Erk1/2 and phosphorylated-STAT3:total-STAT3. However, inhibition of SHP2 activation with PHPS1 treatment, markedly decreased this Hem/CLP-induced rise.

Discussion

These findings demonstrate that, hemorrhagic shock followed by sepsis can induce significant kidney injury as well as lung injury as we have previously documented (Ayala et al. 2002). This study also provides some of the first evidence demonstrating the therapeutic potential of PHPS1 as a treatment directed against AKI induced by Hem/CLP, as our data revealed that PHPS1 could improve renal function. The mechanism we would propose to account for protective effect of PHPS1 involves alleviation of systemic and localized renal inflammation, as evidenced by significantly diminished serum inflammatory factors and renal tissue expression of HMGB1. Further, these effects may be mediated through the inhibition of the Erk1/2-STAT3 signaling pathways.

Many animal models have been used to investigate the development of AKI, including ischemia/reperfusion injury induced AKI (I/R-AKI), direct bacterial or endotoxin administration and CLP-induced sepsis associated acute kidney injury (SA-AKI) (Poston and Koyner 2019). However, while these animal models have been useful for elucidating the mechanism(s) that underpin the development of AKI, they have some limitations. To a certain degree, these models are not in line with clinical pathophysiological processes. In previous studies (Ayala, et al. 2002), we did not observe overt evidence of injury to the lung or kidney in response to hemorrhage or CLP model alone. However, once produced in a sequential fashion, Hem followed CLP, indices of lung injury were clearly induced (Ayala, et al. 2002)

and that appears to also be the case for AKI based on this study. In this respect, patients suffering from trauma often have significant blood loss and receive fluid during recovery in emergency room. And while hemostatic supportive care is critical during this initial period, the impact of this initial event on patient's capacity to handle subsequent insults, infectious or otherwise, can be significant, culminating in multiple organ failure and death in the most severe cases. Using a rodent model of Hem in combination with a subsequent septic challenge, which approximates aspects of what is seen in traumatic shock patients, our laboratory has shown that injection of PHPS1, a specific inhibitor of SHP2, attenuates Hem/CLP-induced AKI in mice.

The systemic inflammatory response may be one of the most important pathogenic factors in this double-hit model. The sharp drop in blood pressure, caused by traumatic shock, is suggested to result in a hypoperfusion in kidney and oliguria, which are thought to induce primarily kidney injury, systemic vasodilatation and oxygen-use abnormalities (Anderson and Watson 2013, Bonventre and Yang 2011). However, excessive administration and accumulation of fluids in an attempt to treat hypotension or oliguria after AKI, is common and also harmful (Boyd et al. 2011, Hjortrup et al. 2016). This may be a signal to trigger the onset of multiple organ failure, even though there is no significant source of infection. Thus, when the secondary septic challenge ensues, it induces a state of systemic inflammation/SIRS and anaerobic metabolism. As shown in our data, many inflammatory factors, including IL-6, TNF- α , IL-10, and chemokines KC and MIP-2, in blood were significantly increased in Hem/CLP group mice. Alleviation of the systemic and localized kidney inflammation appeared to coincide with a reduction in the evidenced of renal tissue injury, i.e., decreased renal tissue NGAL levels and kidney pathologic score. Some studies have revealed that HMGB1 is involved in the pathogenesis of renal disease and have suggested that inhibiting the release of HMGB1 could exert a protective effect against developing AKI (Ruan et al. 2014). A clinical study showed that patients with AKI had higher serum HMGB1 levels (Zakiyanov et al. 2013) and an animal study also reported upregulated expression of mouse renal tissue HMGB1 levels in a model of experimental AKI (Wu et al. 2010). Here we also observed an increased expression of HMGB1 in mouse renal tissue in the double-hit group, and SHP2 inhibition reduced the rise.

The mechanism by which SHP2 inhibition protects against double hit induced AKI remains poorly understood. SHP2, encoded by the PTPN11, plays a significant role in maintaining homeostasis during inflammation by regulating many facets of cell signaling. Our data indicate that injection of SHP2 inhibitor PHPS1 attenuated the release of inflammatory cytokines and renal tissue HMGB1 levels. Previous studies have revealed that select inflammatory signaling pathways are involved in the processes driving AKI. Teng et al. (Teng, et al. 2018) showed that the lentivirus-mediated silencing of SHP2 improved I/R-AKI via inhibition of the TLR4/NF- κ B Pathway. Chen et al. (Chen, et al. 2018) revealed that the SHP2 inhibitor PHPS1 exerted a protective effect against atherosclerosis via suppression of the SHP2:ERK pathway activation. Our current study indicates that SHP2 suppression significantly reduced the levels of phosphorylated Erk1/2 and STAT3 in kidney. This is important as Erk1/2 and STAT3 signaling pathways are thought to be widely involved in the course of inflammation.

That said; there are several limitations to the current study, which should be considered. First, we found an amelioration of systemic and local organ inflammation by SHP2 blockade, however, what the cellular target(s) are involved in the course of mediating the SHP2 to activation of Erk 1/2 and/or STAT3 signaling is still unclear. In previous studies, our laboratory found SHP1 negatively modulated PD-L1 dependent regulation of Treg cell function during the resolution of shock/sepsis-induced lung injury (Tang, et al. 2015). As a component of co-inhibitory receptor signaling sequence of PD-1, how SHP2 recruitment actually suppresses T-cell function in Hem/CLP is incompletely understood. Further studies will be needed to confirm the phenotypic changes that occur at a cellular level in leukocytes from Hem, CLP or Hem/CLP mice. Secondly, while we chose to use PHPS1 to selectively inhibit SHP2 phosphorylation/activation here, it has been reported that when activation of SHP2 by many stimuli include IL-1 and IL-6, growth factors (insulin, EGF, FGF, etc.), they produced a deleterious effect in cancer (Zhang et al. 2015), which is supportive of our findings. Additionally, different species have different sensitivity to such agents, so it is unclear whether PHPS1 would necessarily have a similar positive effect in the clinical trauma patient. Inasmuch; we feel these data indicate that further clinical research should be done to establish if such issues exist. Finally, Erk1/2 and STAT3 signaling pathway are both involved in mediating IL-6 inflammatory signaling, however, IL-6 levels in our model were not effected by PHPS1 treatment. This suggests, there may be other cytokines involved in the course of this double hit induced kidney injury that are mediating the effects of PHPS1 inhibition in the kidney. Perhaps hemorrhagic shock induced noninfectious inflammation is a critical contributory factor in this model, however, its precise mechanism of action is still unclear. Despite this, our data also indicates that the inhibition of Erk1/2 and/or STAT3 signaling may be the key aspects of the protective actions of PHPS1 treatment against Hem/CLP induce AKI.

Conclusion

In conclusion, our data support the tenet that SHP2 inhibition attenuates AKI-induced by our double-hit/sequential insult model of Hem/CLP and that this protective action may be attributable to its anti-inflammatory property in mitigating activation of the Erk1/2 and STAT3 signaling pathway. This finding we believe has important potential clinical implications and, thus, warrants further investigation.

Abbreviations

Acute kidney injury (AKI); Blood urea nitrogen (BUN); Cecal ligation and puncture (CLP); Creatinine (Cre); Enzyme linked immune-assay (ELISA); Epidermal growth factor (EGF); Extracellular-regulated kinase (Erk1/2); Fibroblast growth factor (FGF); Glyceraldehyde 3-phosphate dehydrogenase (GAPDH); Growth factor receptor-bound protein 2 (Grb-2)–associated binder 1 (Gab1); Hematoxylin and eosin (HE); Hemorrhage (Hem); Hemorrhage followed by cecal ligation and puncture mice (Hem/CLP); High mobility group box 1 (HMGB1); Intracellular tyrosine inhibitory motif (ITIM); Intracellular tyrosine switch motif (ITSM); Interleukin-6 (IL-6); Interleukin-10 (IL-10); Ischemia/reperfusion injury-AKI (I/R-AKI); Keratinocyte chemoattractant (KC); Kidney/body weight index (KI); Macrophage inflammatory protein-2 (MIP-2);

Neutrophil gelatinase-associated lipocalin (NGAL); Nuclear Factor-kappa beta (NF-kB); Phenylhydrazonopyrazolone sulfonate 1 (PHPS1); Phosphatase protein tyrosine phosphatase (PTPs); Phosphatidylinositol (PI); Programmed cell death receptor-1 (PD-1); Programmed cell death receptor-ligand1 (PD-L1); Sham-Hem followed by sham-CLP mice (S/S); Sepsis associated-AKI (SA-AKI); Src homology 2 domain-containing phosphatase 2 (SHP2); Signal transducer and activator of transcription 3 (STAT3); Systemic inflammatory response syndrome (SIRS); T regulatory lymphocyte (Treg); Toll-like receptor-4 (TLR4); Tumor necrosis factor (TNF- α);

Declarations

Ethical Approval and Consent to participate: All experiments were performed in accordance with National Institutes of Health guidelines and approved by the Animal Use Committee of Rhode Island Hospital (AWC# 5064-18). There where no human subjects studies done here.

Consent for publication: This manuscript is solely submitted to *Molecular Medicine* and is not under consideration at any other journal nor has any part been previously published.

Conflict of Interest: It should also be noted that all authors concur with the material submitted in this manuscript and that none of the authors have any financial interests or affiliations with commercial organizations whose products or services are related to the subject matter of this manuscript (no existing conflicts of interest).

Availability of supporting data: The data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

Competing interests: The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Funding: This work was supported by NIH R35-GM118097 (A.A.), as well as a fellowship support from Shanghai General Hospital (J.J.) & Shanghai Pudong Hospital (B.H.).

Author Contributions:

Conception and study design: Jiang, Hu, Chun, Li, Ayala

Acquisition of data: Jiang, Hu, Zhang, Chen, Chung

Analysis and/or interpretation of data: Jiang, Hu, Zhang, Chen, Chung

Drafting the manuscript: Jiang, Hu, Zhang, Chung

Revising the manuscript for important intellectual content: Jiang, Hu, Chung, Li, Ayala

Acknowledgements: Histological processing for H&E staining was supported by the Molecular Pathology Core of the COBRE Center for Cancer Research Development at Rhode Island Hospital, funded by the NIH under award number P30-GM110759/P20-GM103421/P20-RR017695.

References

1. Anderson MW, Watson GA. Traumatic shock: the fifth shock. *J Trauma Nurs.* 2013;20:37–43.
2. Ayala A, Chung CS, Lomas JL, Song GY, Doughty LA, Gregory SH, et al. Shock-induced neutrophil mediated priming for acute lung injury in mice: divergent effects of TLR-4 and TLR-4/FasL deficiency. *Am J Pathol.* 2002;161:2283–94.
3. Barbar SD, Clere-Jehl R, Bourredjem A, Hernu R, Montini F, Bruyere R, et al. Timing of Renal Replacement Therapy in Patients with Acute Kidney Injury and Sepsis. *N Engl J Med.* 2018;379:1431–42.
4. Biron BM, Chung CS, Chen Y, Wilson Z, Fallon EA, Reichner JS, et al. PAD4 Deficiency Leads to Decreased Organ Dysfunction and Improved Survival in a Dual Insult Model of Hemorrhagic Shock and Sepsis. *J Immunol.* 2018;200:1817–28.
5. Bonventre JV, Yang L. Cellular pathophysiology of ischemic acute kidney injury. *The Journal of clinical investigation.* 2011;121:4210–21.
6. Boyd JH, Forbes J, Nakada TA, Walley KR, Russell JA. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. *Crit Care Med.* 2011;39:259–65.
7. Chen J, Cao Z, Guan J. SHP2 inhibitor PHPS1 protects against atherosclerosis by inhibiting smooth muscle cell proliferation. *BMC Cardiovasc Disord.* 2018;18:72.
8. Chichger H, Braza J, Duong H, Harrington EO. SH2 domain-containing protein tyrosine phosphatase 2 and focal adhesion kinase protein interactions regulate pulmonary endothelium barrier function. *American journal of respiratory cell molecular biology.* 2015;52:695–707.
9. Dellepiane S, Marengo M, Cantaluppi V. Detrimental cross-talk between sepsis and acute kidney injury: new pathogenic mechanisms, early biomarkers and targeted therapies. *Crit Care.* 2016;20:61.
10. Gomez H, Ince C, De Backer D, Pickkers P, Payen D, Hotchkiss J, et al. A unified theory of sepsis-induced acute kidney injury: inflammation, microcirculatory dysfunction, bioenergetics, and the tubular cell adaptation to injury. *Shock.* 2014;41:3–11.
11. Hellmuth K, Grosskopf S, Lum CT, Wurtele M, Roder N, von Kries JP, et al. Specific inhibitors of the protein tyrosine phosphatase Shp2 identified by high-throughput docking. *Proc Natl Acad Sci U S A.* 2008;105:7275–80.
12. Hjortrup PB, Haase N, Bundgaard H, Thomsen SL, Winding R, Pettila V, et al. Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. *Intensive Care Med.* 2016;42:1695–705.

13. Honore PM, Jacobs R, Hendrickx I, Bagshaw SM, Joannes-Boyau O, Boer W, et al. Prevention and treatment of sepsis-induced acute kidney injury: an update. *Ann Intensive Care*. 2015;5:51.
14. Hsu MF, Bettaieb A, Ito Y, Graham J, Havel PJ, Haj FG. Protein tyrosine phosphatase Shp2 deficiency in podocytes attenuates lipopolysaccharide-induced proteinuria. *Scientific reports*. 2017;7:461.
15. Karasu E, Nilsson B, Kohl J, Lambris JD, Huber-Lang M. Targeting Complement Pathways in Polytrauma- and Sepsis-Induced Multiple-Organ Dysfunction. *Front Immunol*. 2019;10:543.
16. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26:677–704.
17. Kellum JA, Wen X, de Caestecker MP, Hukriede NA. Sepsis-Associated Acute Kidney Injury: A Problem Deserving of New Solutions. *Nephron*. 2019;143:174–8.
18. Kellum JA, Wen X, de Caestecker MP, Hukriede NA. Sepsis-Associated Acute Kidney Injury: A Problem Deserving of New Solutions. *Nephron*. 2019: 1–5.
19. Maroun CR, Naujokas MA, Holgado-Madruga M, Wong AJ, Park M. The tyrosine phosphatase SHP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the met receptor tyrosine kinase. *Mol Cell Biol*. 2000;20:8513–25.
20. Patil NK, Guo Y, Luan L, Sherwood ER. Targeting Immune Cell Checkpoints during Sepsis. *Int J Mol Sci*. 2017;18.
21. Poston JT, Koyner JL. Sepsis associated acute kidney injury. *BMJ (Clinical research ed)*. 2019;364:k4891.
22. Riley JL. PD-1 signaling in primary T cells. *Immunol Rev*. 2009;229:114–25.
23. Ruan Y, Wang L, Zhao Y, Yao Y, Chen S, Li J, et al. Carbon monoxide potently prevents ischemia-induced high-mobility group box 1 translocation and release and protects against lethal renal ischemia-reperfusion injury. *Kidney Int*. 2014;86:525–37.
24. Spahn DR, Bouillon B, Cerny V, Duranteau J, Filipescu D, Hunt BJ, et al. The European guideline on management of major bleeding and coagulopathy following trauma: fifth edition. *Crit Care*. 2019;23:98.
25. Tang L, Bai J, Chung CS, Lomas-Neira J, Chen Y, Huang X, et al. Programmed cell death receptor ligand 1 modulates the regulatory T cells' capacity to repress shock/sepsis-induced indirect acute lung injury by recruiting phosphatase SRC homology region 2 domain-containing phosphatase 1. *Shock*. 2015;43:47–54.
26. Teng JF, Wang K, Jia ZM, Guo YJ, Guan YW, Li ZH, et al. Lentivirus-Mediated Silencing of Src Homology 2 Domain-Containing Protein Tyrosine Phosphatase 2 Inhibits Release of Inflammatory Cytokines and Apoptosis in Renal Tubular Epithelial Cells Via Inhibition of the TLR4/NF- κ B Pathway in Renal Ischemia-Reperfusion Injury. *Kidney Blood Press Res*. 2018;43:1084–103.
27. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. *JAMA*. 2005;294:813–8.

28. Verma R, Venkatarreddy M, Kalinowski A, Patel SR, Salant DJ, Garg P. Shp2 Associates with and Enhances Nephritin Tyrosine Phosphorylation and Is Necessary for Foot Process Spreading in Mouse Models of Podocyte Injury. *Mol Cell Biol.* 2016;36:596–614.
29. Vijayakumar EC, Bhatt LK, Prabhavalkar KS. High Mobility Group Box-1 (HMGB1): A potential target in therapeutics. *Curr Drug Targets.* 2019.
30. Wakeley ME, Gray CC, Monaghan SF, Heffernan DS, Ayala A. Check Point Inhibitors and Their Role in Immunosuppression in Sepsis. *Crit Care Clin.* 2020;36:69–88.
31. Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. *American journal of respiratory critical care medicine.* 2001;164:1768–73.
32. White LE, Hassoun HT, Bihorac A, Moore LJ, Sailors RM, McKinley BA, et al. Acute kidney injury is surprisingly common and a powerful predictor of mortality in surgical sepsis. *J Trauma Acute Care Surg.* 2013;75:432–8.
33. Wu H, Ma J, Wang P, Corpuz TM, Panchapakesan U, Wyburn KR, et al. HMGB1 contributes to kidney ischemia reperfusion injury. *J Am Soc Nephrol.* 2010;21:1878–90.
34. Wu Y, Wang L, Meng L, Cao GK, Zhao YL, Zhang Y. Biological effects of autophagy in mice with sepsis-induced acute kidney injury. *Experimental therapeutic medicine.* 2019;17:316–22.
35. Zakiyanov O, Kriha V, Vachek J, Zima T, Tesar V, Kalousova M. Placental growth factor, pregnancy-associated plasma protein-A, soluble receptor for advanced glycation end products, extracellular newly identified receptor for receptor for advanced glycation end products binding protein and high mobility group box 1 levels in patients with acute kidney injury: a cross sectional study. *BMC Nephrol.* 2013;14:245.
36. Zhang J, Zhang F, Niu R. Functions of Shp2 in cancer. *J Cell Mol Med.* 2015;19:2075–83.
37. Zhang SQ, Tsiaras WG, Araki T, Wen G, Minichiello L, Klein R, et al. Receptor-specific regulation of phosphatidylinositol 3'-kinase activation by the protein tyrosine phosphatase Shp2. *Mol Cell Biol.* 2002;22:4062–72.
38. Zhang Z, Zhao H, Ge D, Wang S, Qi B. beta-Casomorphin-7 Ameliorates Sepsis-Induced Acute Kidney Injury by Targeting NF-kappaB Pathway. *Medical science monitor: international medical journal of experimental clinical research.* 2019;25:121–7.

Figures

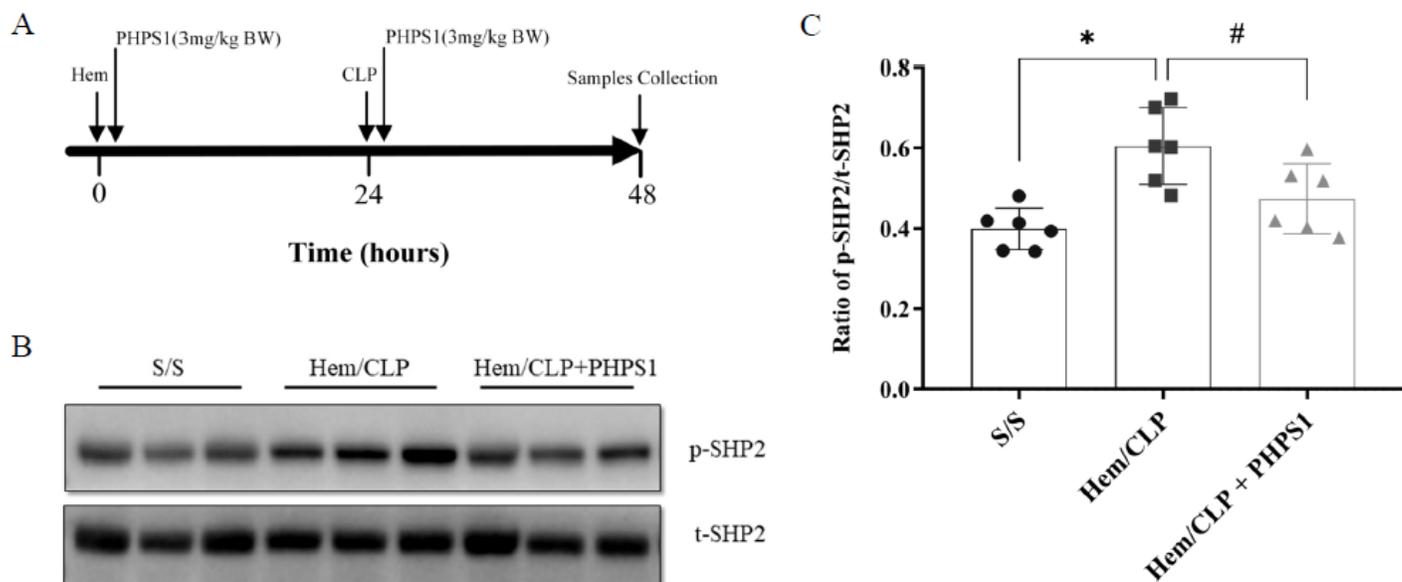


Figure 1

SHP2 activation in the kidney reduced by PHPS1 treatment. Diagram of experimental timeline for PHPS1 (3mg/kg BW) administration, Hem, CLP and mice sacrifice (A). Twenty four h post-Hem/CLP or S/S operation, the kidneys were collected and tissue homogenates were obtained. The extent of SHP2 activation (phosphorylation) was determined by western blot (B-C). The ratio of phosphorylated SHP2 and total SHP2 (p-SHP2/t-SHP2) in kidneys from Hem/CLP mice was significantly increased compared to S/S. PHPS1 treated Hem/CLP mice showed reduced SHP2 activation compared to vehicle treated Hem/CLP mice. *, $P < 0.05$, versus (S/S); #, $P < 0.05$, PHPS1 treated group (Hem/CLP + PHPS1) versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; n=6 mice/group.

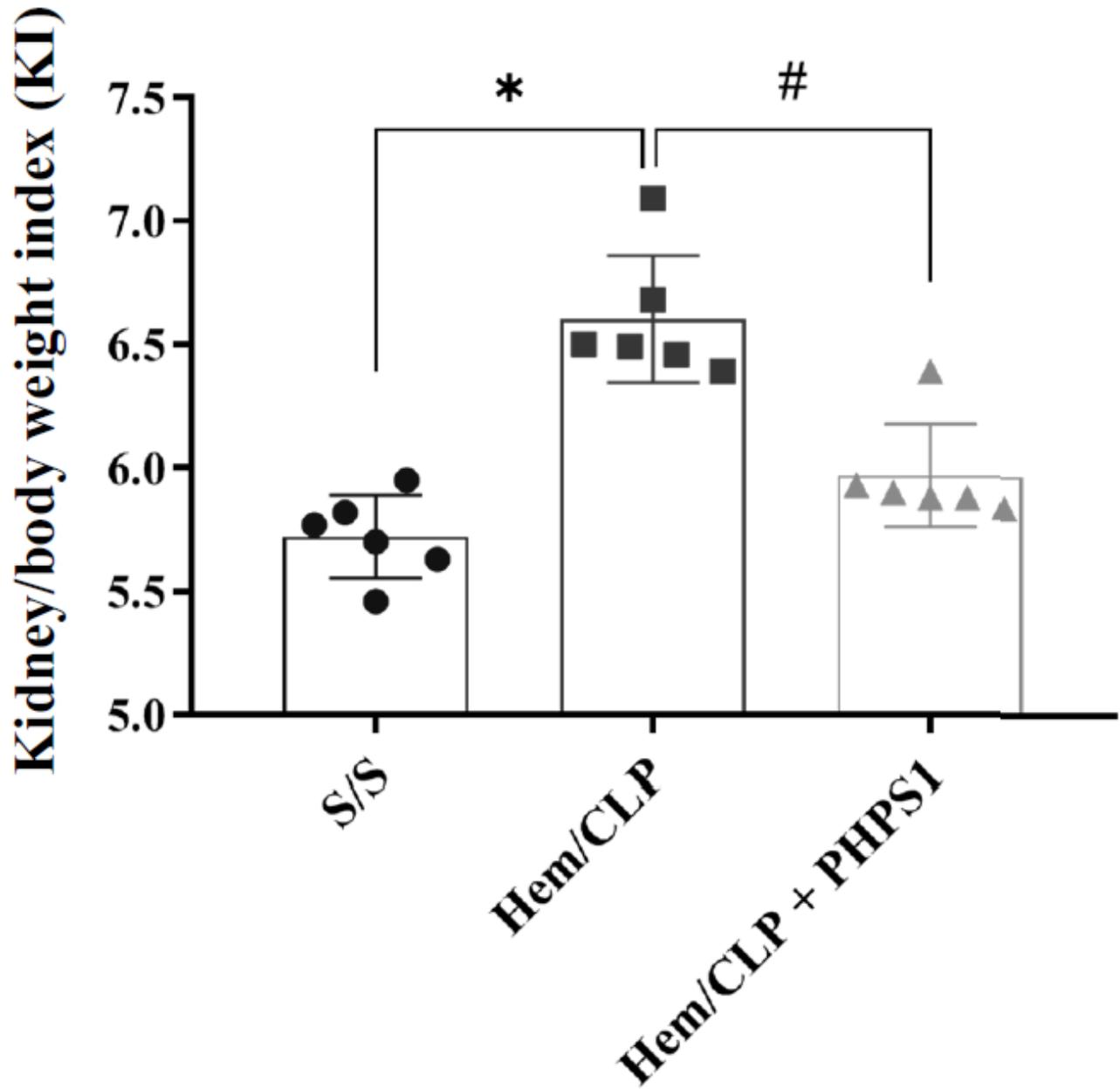


Figure 2

PHPS1 treatment decreased kidney/body weight index (KI) elevated by Hem/CLP. Twenty four hours post Hem/CLP or S/S operation, body weight were measured and the kidneys were collected and weighted. Vehicle treated Hem/CLP mice showed increased KI compared to S/S and PHPS1 treated Hem/CLP mice. *, P<0.05, versus S/S; #, P<0.05, PHPS1 treated group (Hem/CLP + PHPS1) versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; n=6 mice/group.

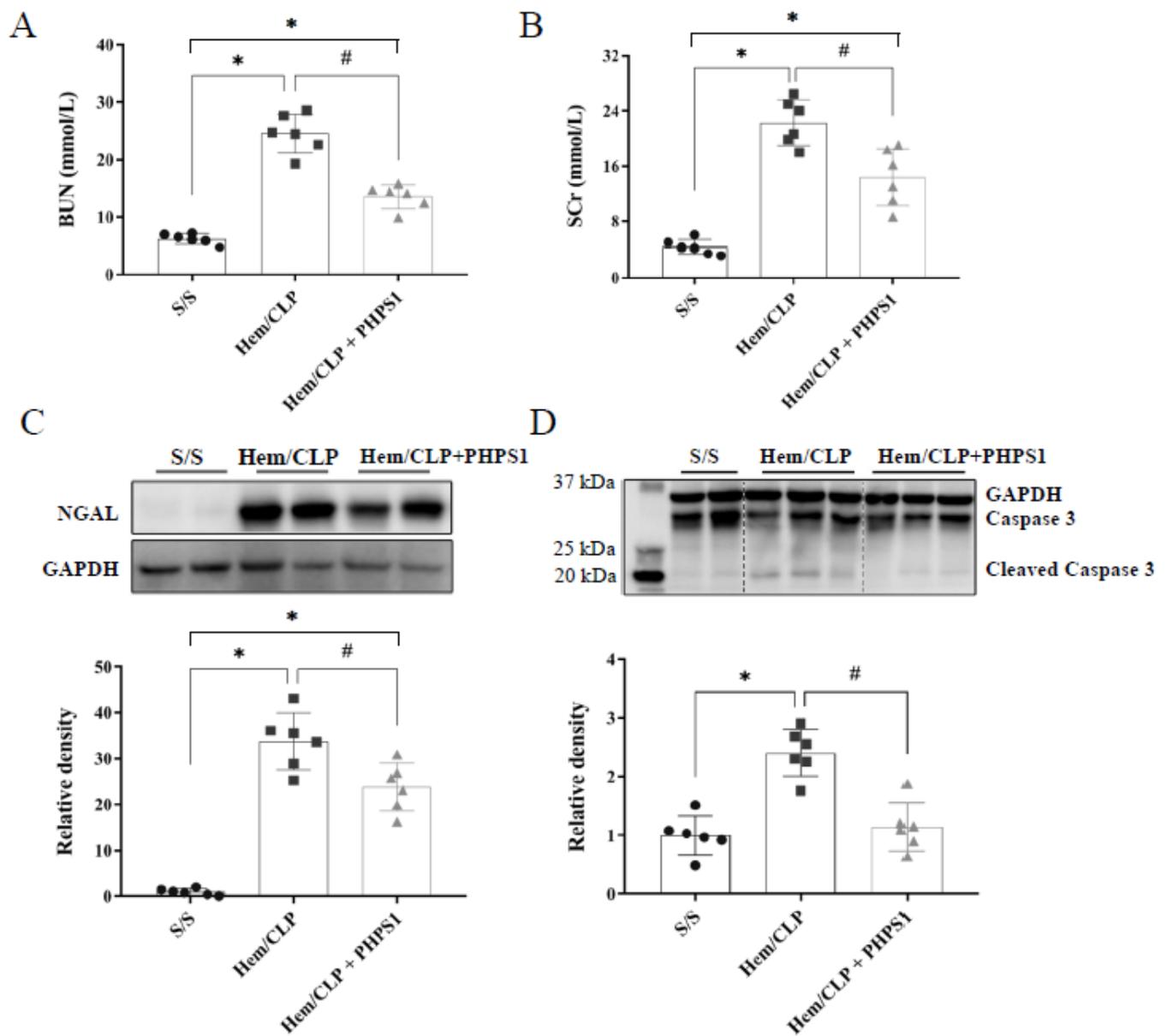


Figure 3

Effects of SHP2 inhibition by PHPS1 treatment on kidney injury after Hem/CLP. Twenty four hours post Hem/CLP or S/S operation, the plasma samples were collected for urea nitrogen (BUN) (A), creatinine (SCr) (B). Kidney tissues were collected for NGAL (C) and cleaved caspase 3 levels (D) by western blot analysis. Vehicle treated Hem/CLP mice showed significantly increased in BUN, SCr, NGAL and cleaved caspase 3 levels compared to S/S mice. However, these increases were markedly reduced in PHPS1 treated Hem/CLP mice. *, $P < 0.05$, versus S/S; #, $P < 0.05$, PHPS1 treated group (Hem/CLP + PHPS1) versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; $n = 6$ mice/group.

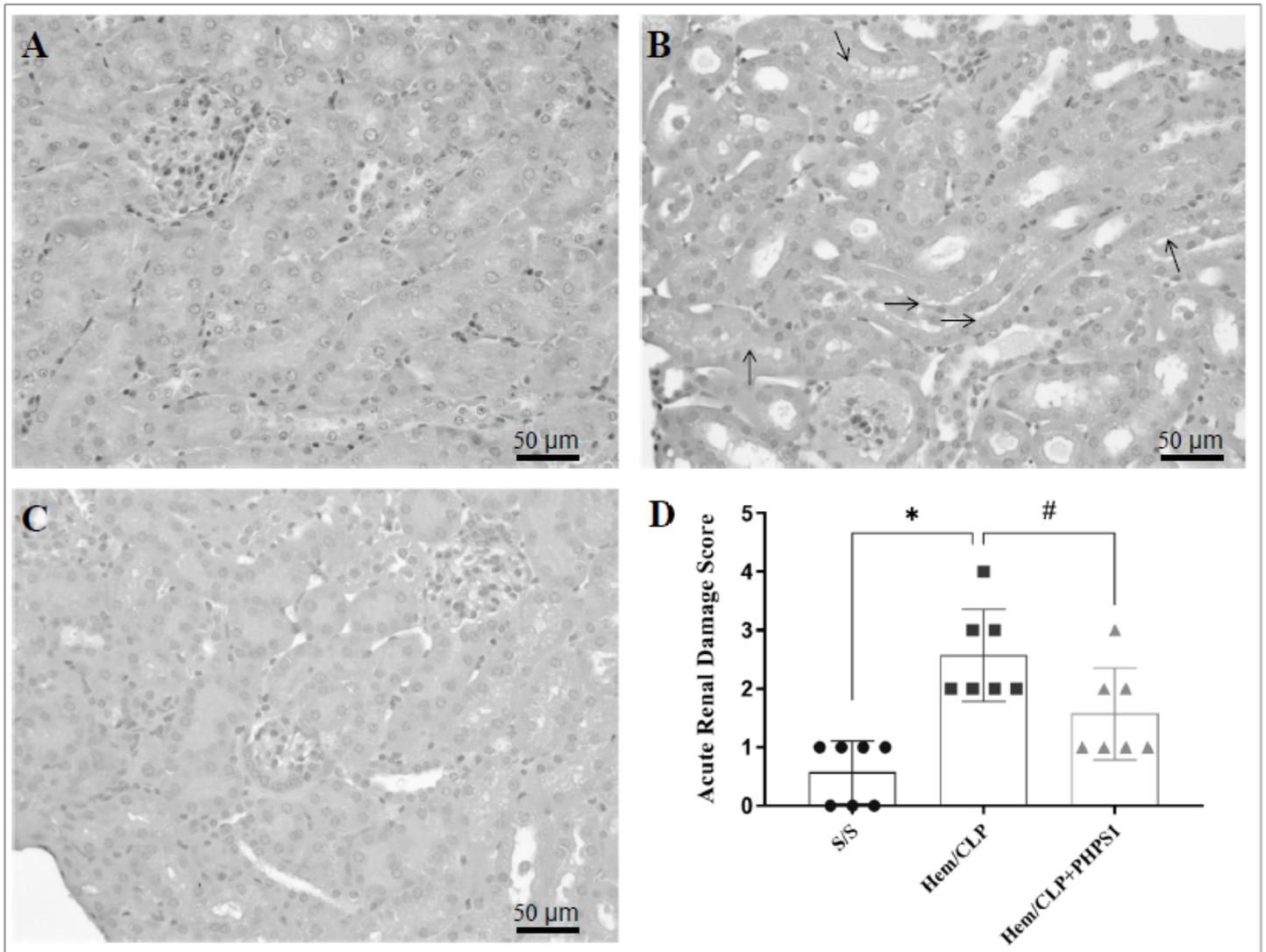


Figure 4

Effects of SHP2 inhibition on kidney histopathologic changes after Hem/CLP. The kidneys were collected twenty four hours post Hem/CLP or S/S operation. Representative H&E staining of kidney sections for detection of injury by pathological changes. Compared to S/S group (A), the kidney from Hem/CLP mice (B) exhibited tubular epithelial cell sloughing/detachment (black arrows), edema, inflammatory cell infiltration, loss of brush borders, tubular dilation, and tubular distortion, examined under light microscopy (20X) (scale bar: 50 μ m). However, PHPS1 treatment markedly ameliorated these changes and preserved the renal architecture (C). The summary data of injury score of 3 to 5 fields/slide/sample at 200x magnification (D). *, $P < 0.05$, versus S/S; #, $P < 0.05$, PHPS1 treated group (Hem/CLP + PHPS1) versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; $n = 6$ mice/group.

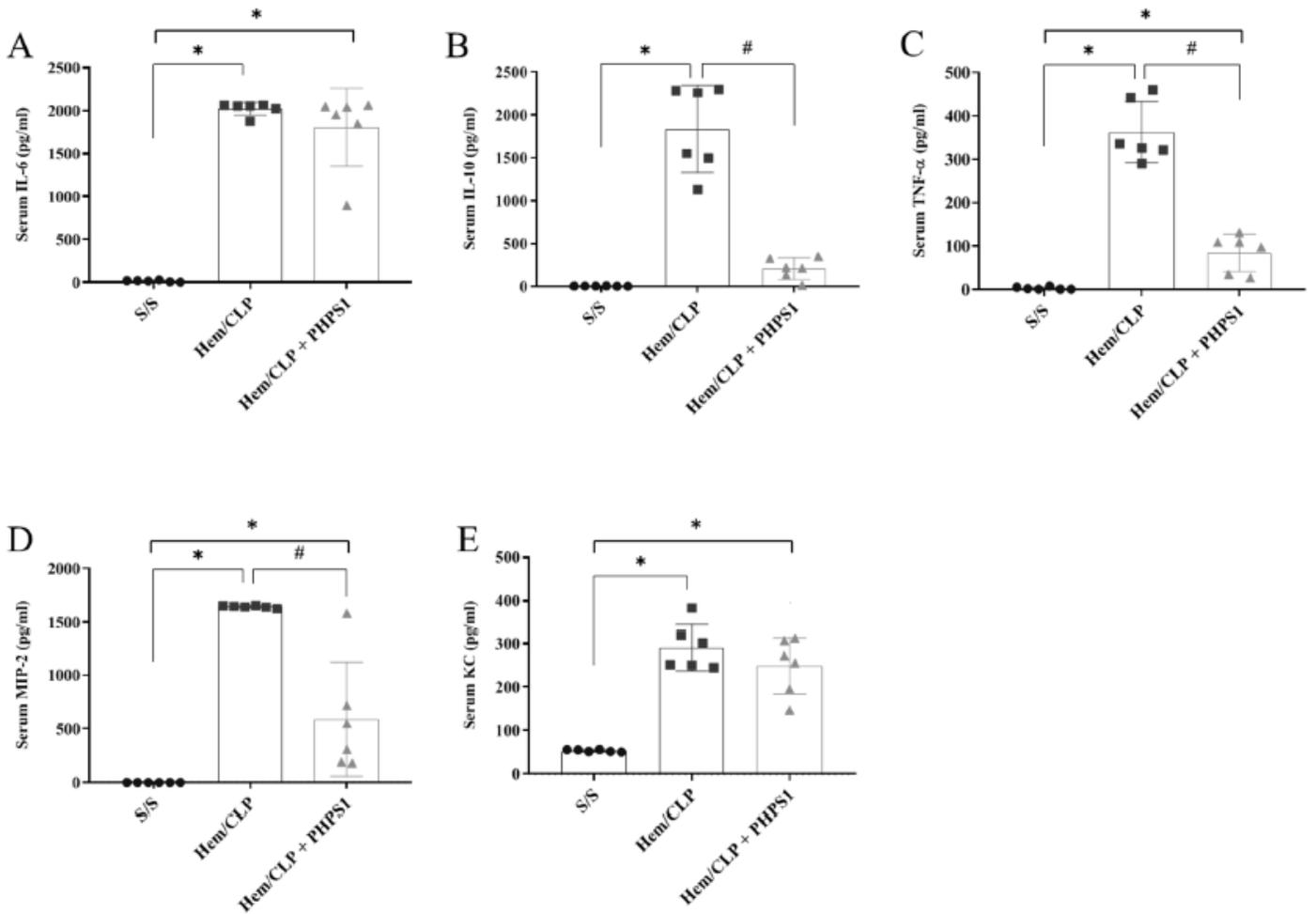


Figure 5

Effects of PHPS1 on systemic plasma levels of inflammatory cytokine/chemokine produced after Hem/CLP. The plasma levels of IL-6 (A), IL-10 (B), TNF- α (C), MIP-2 (D) and KC (E) in the vehicle or the PHPS1 treated Hem/CLP mice were increased compared to the S/S animals. However, this Hem/CLP-induced increase was markedly suppressed by PHPS1 treatment for IL-10, TNF- α and MIP-2, but not IL-6 and KC levels. * $P < 0.05$, versus Sham (S/S); # $P < 0.05$, PHPS1 treated group (Hem/CLP + PHPS1) versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; $n = 6$ mice/group.

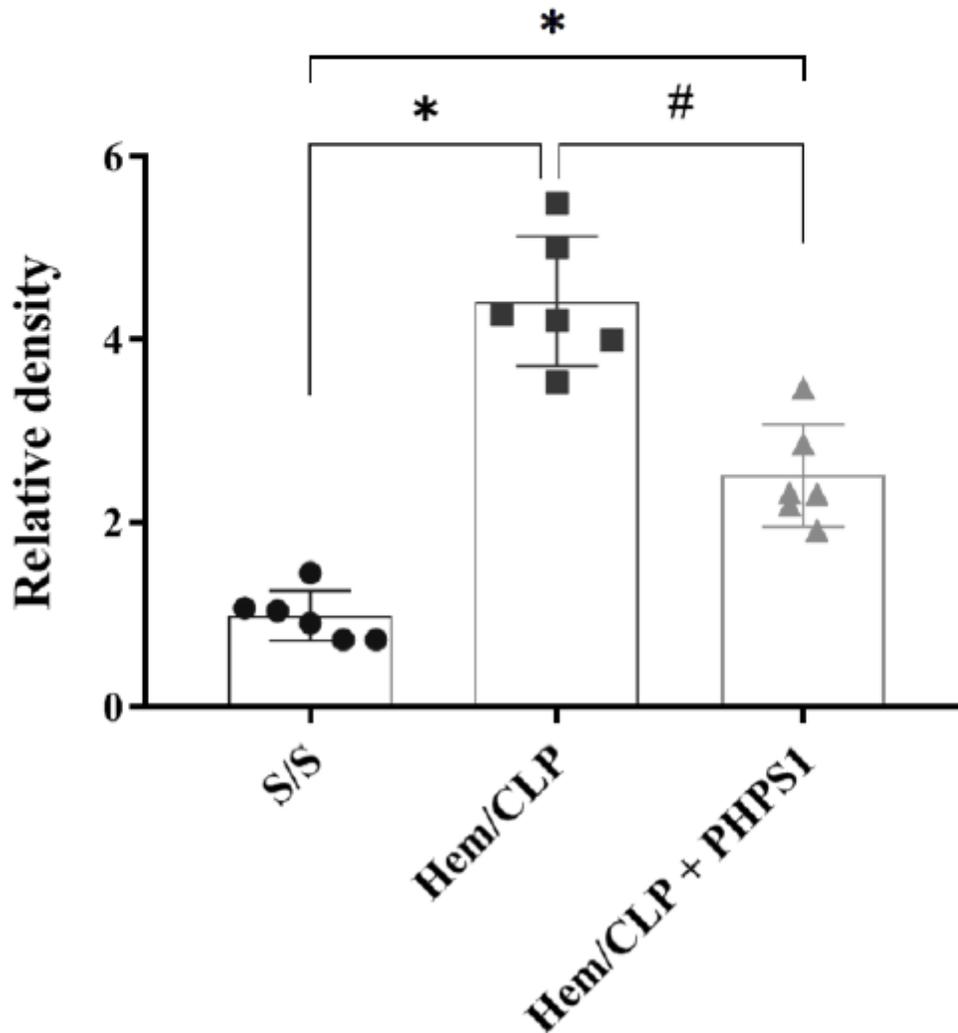
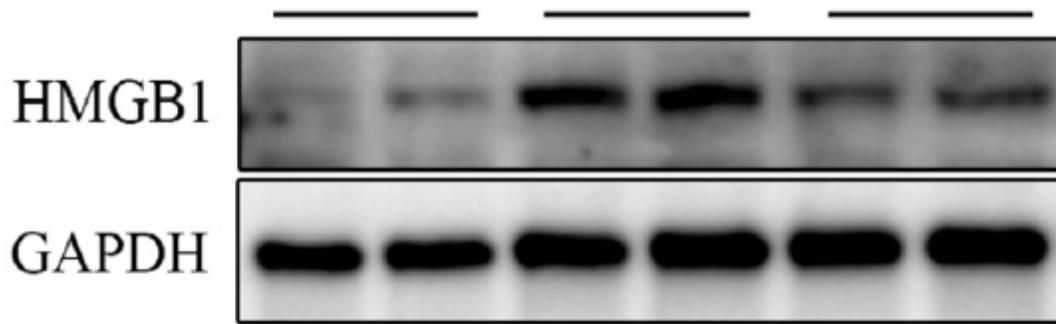


Figure 6

Effects of PHPS1 on expression of HMGB1 in kidney after Hem/CLP. The kidneys were collected twenty four hours post Hem/CLP or S/S operation and tissue homogenates were obtained. The expression of HMGB1 was determined by western blot. The levels of HMGB1 from Hem/CLP mice was significantly increased compared to S/S. PHPS1 treated Hem/CLP mice showed reduced HMGB1 levels compared to Veh treated HEM/CLP mice. *, $P < 0.05$, versus S/S; #, $P < 0.05$, PHPS1 treated group (Hem/CLP + PHPS1)

versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; n=6 mice/group.

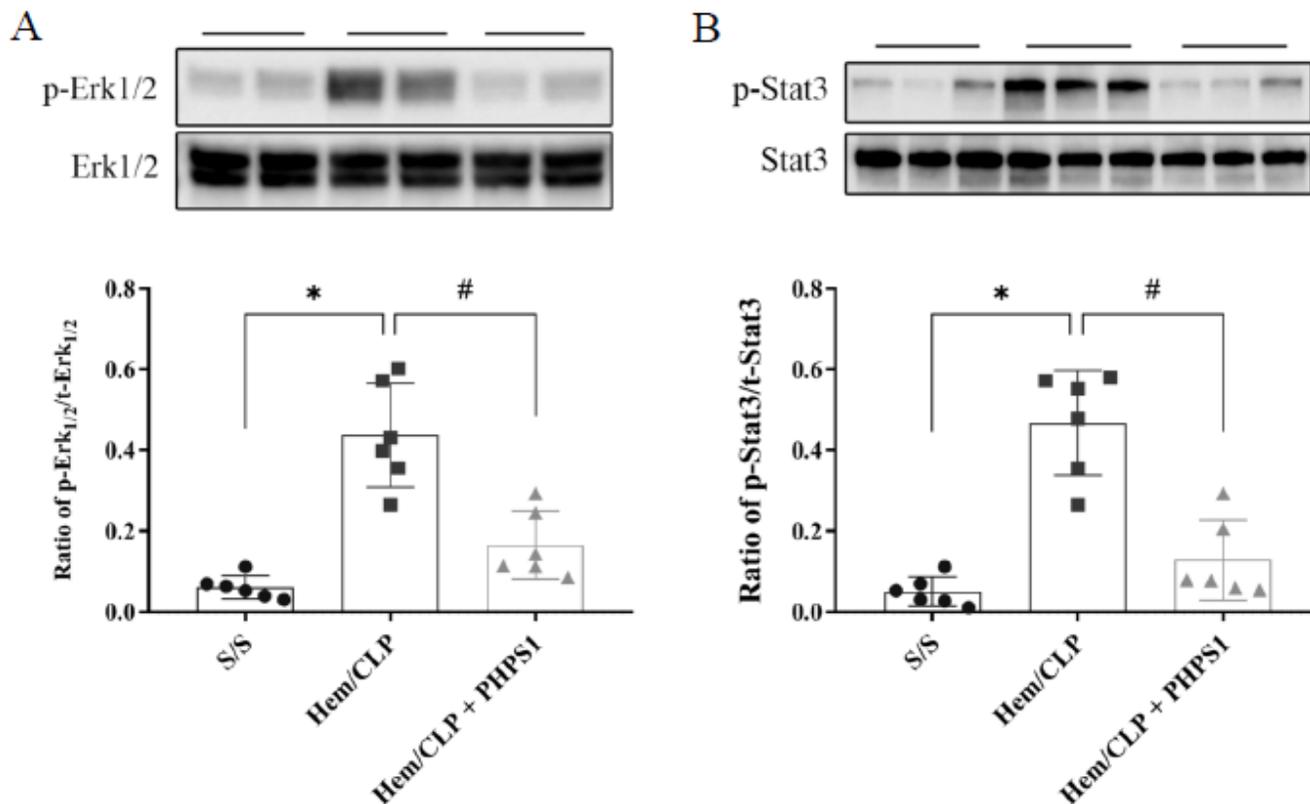


Figure 7

Effects of PHPS1 on expression of HMGB1 in kidney after Hem/CLP. The kidneys were collected twenty four hours post Hem/CLP or S/S operation and tissue homogenates were obtained. The expression of HMGB1 was determined by western blot. The levels of HMGB1 from Hem/CLP mice was significantly increased compared to S/S. PHPS1 treated Hem/CLP mice showed reduced HMGB1 levels compared to Veh treated HEM/CLP mice. *, P<0.05, versus S/S; #, P<0.05, PHPS1 treated group (Hem/CLP + PHPS1) versus Hem/CLP+vehicle (Hem/CLP). One-way ANOVA and a Student-Newman-Keuls' test, Mean \pm SD; n=6 mice/group.