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## Propofol increases bone morphogenetic protein-7 and decreases oxidative stress in sepsis-induced acute kidney injury

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### Abstract

**Background.** Pro-inflammatory cytokines and free radicals damage renal tissue leading to acute kidney injury (AKI) during sepsis. Bone morphogenetic protein-7 (BMP-7) represses tumour necrosis factor (TNF)- $\alpha$ -induced inflammatory responses and protects kidney from injury. The sedative agent, propofol, has immunomodulatory and antioxidative properties. The present study investigated whether propofol could reduce AKI in caecal ligation and puncture (CLP) mice and the possible mechanism behind this.

**Methods.** Mice were treated with propofol or saline immediately and 12 h after CLP surgery. Kidney injury, survival and cytokine expressions of CLP mice were observed 24 h after CLP surgery. *In vitro*, lipopolysaccharide (LPS)-stimulated rat mesangial cells (RMCs) or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-exposed murine kidney epithelial cells (M1) were treated with propofol. The expression of BMP-7, TNF- $\alpha$  and monocyte chemotactic protein (MCP)-1 in CLP mice kidney, RMCs or M1 cells was determined by RT-PCR. Free radical generation and cell death of RMCs and M1 cells were analysed. Nuclear factor (NF)- $\kappa$ B and peroxisome proliferator-activated receptor (PPAR)- $\gamma$  expressions in LPS-stimulated RMCs were determined by western blotting.

**Results.** Propofol increased survival and ameliorated AKI in CLP mice. Propofol increased BMP-7 expression but decreased TNF- $\alpha$  and MCP-1 expressions in the kidney of CLP mice and LPS-stimulated RMCs. Propofol also inhibited free radical generation and cell death in LPS-stimulated RMCs and decreased the TNF- $\alpha$  expression and cell death in H<sub>2</sub>O<sub>2</sub>-exposed M1 cells. Moreover, propofol decreased NF- $\kappa$ B but increased PPAR- $\gamma$  expression in LPS-stimulated RMCs.

**Conclusions.** Propofol treatment could protect kidney from sepsis-induced AKI by increasing BMP-7 expression, decreasing inflammatory cytokines and inhibiting oxidative stress.

**Keywords:** acute kidney injury; bone morphogenetic protein-7; oxidative stress; propofol; sepsis

### Introduction

Sepsis is characterized by the systemic inflammatory response to infection and is associated with multiple organ failure [1]. Sepsis-induced acute kidney injury (AKI) is as-

sociated with 70% of mortality, and is a serious medical problem in the intensive care unit [2]. The pro-inflammatory responses including formation of chemotactic factors, neutrophil recruitment, cytokines release and free radical generation all contribute to oxidative stress thus leading to sepsis-induced AKI [3–5]. *In vivo* and *in vitro* studies demonstrated that exposure of animals or renal cells to lipopolysaccharide (LPS) induces inflammatory responses and free radical, including reactive oxygen species (ROS) and nitric oxide (NO), generation. Antioxidants can protect against AKI caused by oxidative stress in murine models of endotoxaemia [2]. Inhibiting free radical generation may influence the LPS-induced septic pathogenesis [6,7]. Despite a growing understanding of the pathophysiological mechanisms of sepsis-induced AKI, pharmacological means for preventing AKI during sepsis are relative slow [8]. This study aims to investigate the effect of propofol on preventing kidney injury in sepsis mice and LPS-stimulated renal cells.

LPS activates the nuclear factor (NF)- $\kappa$ B pathway in the kidney leading to inflammatory cytokine synthesis, free radical production and cell death [9,10]. In sepsis-induced AKI, tumour necrosis factor (TNF)- $\alpha$  magnifies the responses mediated by other cytokines and free radical generation through activation of the NF- $\kappa$ B signalling pathway [11–13]. Promoting the activity of peroxisome proliferator-activated receptor (PPAR)- $\gamma$  could decrease TNF- $\alpha$  during sepsis by inhibiting NF- $\kappa$ B activation [14,15]. Another cytokine, bone morphogenetic protein-7 (BMP-7), a member of the TGF- $\beta$  superfamily, is crucial in nephrogenesis during development. BMP-7 can reduce the severity of glomerulonephritis and tubulointerstitial fibrosis in various experimental models of acute or chronic renal injury [16–19]. BMP-7 can repress TNF- $\alpha$ -induced inflammatory responses during nephropathy by inhibiting the activity of NF- $\kappa$ B but increasing the activity of PPAR- $\gamma$  in the IgA-induced nephropathy model [20,21]. Moreover, antioxidant treatment may increase renal BMP-7 expression, which produces positive feedback and improves renal function during diabetic nephropathy [22,23]. Thus, BMP-7 has been suggested as a therapeutic target for the treatment of acute or chronic kidney injuries [24].

Propofol (2,6-diisopropylphenol), containing a phenol hydroxyl group, which confers antioxidant activity, is widely used for the induction and maintenance of general

anaesthesia [25,26]. Propofol treatment could inhibit pro-inflammatory cytokines in septic animal models [27–31] and septic patients [32]. However, the effect of propofol on sepsis-induced AKI and its molecular mechanism remain unclear. In this study, caecal ligation and puncture (CLP)-induced polymicrobial sepsis in mice was used to investigate whether propofol treatment could increase renal BMP-7 expression and prevent kidney from sepsis-induced AKI. The effects of propofol on regulating inflammatory cytokines and inhibiting oxidative stress in LPS-stimulated mesangial cells were clarified.

## Materials and methods

### Animal model: CLP

Male BALB/c mice (7–8 weeks old) had free access to water and standard chow. Mice were anaesthetized with 100 mg/kg ketamine intramuscularly. After laparotomy, a 5-0 silk ligature was placed 5 mm from the caecal tip. The caecum was punctured twice with a 21-gauge needle and gently squeezed to express a 1 mm column of faecal material. In sham-operated mice, the caecum was exposed but was neither ligated nor punctured. Then, the abdominal incision was closed in two layers, and 1 mL of saline was given intraperitoneally (i.p.) to all mice after surgery. To investigate the effect of propofol during sepsis-induced AKI, mice were divided into four groups ( $n = 6$  in each group): (i) normal mice as control, (ii) CLP mice injected with saline, (iii) CLP mice injected with propofol and (iv) sham-operated mice injected with propofol. A subhypnotic dose of propofol 50 mg/kg (Astra Zeneca, London, UK) [33–35] or an equal volume of saline was injected i.p. immediately and 12 h after surgery. Twenty-four hours after CLP surgery, mice were sacrificed, and blood from their abdominal aorta was collected for serum biochemical analysis. The lungs, livers and kidneys of the mice were dissected and stored in 10% formalin or frozen in liquid nitrogen for further analysis. In another experiment, we observed the 48-h survival of CLP mice with or without propofol treatment.

### Serum biochemical analysis

Serum samples from mice were prepared by centrifugation at 12 000 g for 3 min. We analysed the levels of C-reactive protein (CRP), glutamate oxaloacetate transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN) or creatinine using commercial kit reagents (AppliedBio assay kits; Hercules, CA, USA) and an auto-analyser (Quik-Lab, USA). Each sample was run in duplicate.

### Cell culture and reagents

A rat mesangial cell (RMC) line and murine kidney cortical collecting duct epithelial M1 cell line purchased from the American Type Culture Collection (Manassas, USA) were maintained in Dulbecco's modified Eagle's medium supplemented with 10–15% fetal bovine serum. At around 60% confluent, cells were treated with 100 ng/mL LPS (*Escherichia coli*

**Table 1.** Effect of propofol treatment on the serum CRP, GOT, GPT, BUN and creatinine levels in CLP-operated or sham-operated mice with saline or propofol treatment

	Control	CLP	CLP + propofol	Sham + propofol
CRP (mg/dL)	3.45 $\pm$ 0.36 <sup>b</sup>	6.40 $\pm$ 0.70 <sup>a</sup>	4.43 $\pm$ 0.35 <sup>b</sup>	3.20 $\pm$ 0.17 <sup>b</sup>
GOT (U/L)	69.67 $\pm$ 6.25 <sup>b</sup>	733.50 $\pm$ 128.88 <sup>a</sup>	423.33 $\pm$ 87.29	143.00 $\pm$ 29.02 <sup>b</sup>
GPT (U/L)	40.50 $\pm$ 7.72 <sup>b</sup>	193.00 $\pm$ 21.79 <sup>a</sup>	125.33 $\pm$ 26.87	52.00 $\pm$ 7.51 <sup>b</sup>
BUN (mg/dL)	24.73 $\pm$ 0.94 <sup>b</sup>	47.75 $\pm$ 9.84 <sup>a</sup>	22.37 $\pm$ 0.61 <sup>b</sup>	24.87 $\pm$ 0.70 <sup>b</sup>
Creatinine ( $\mu$ g/dL)	0.17 $\pm$ 0.05 <sup>b</sup>	0.88 $\pm$ 0.10 <sup>a</sup>	0.27 $\pm$ 0.07 <sup>b</sup>	0.24 $\pm$ 0.02 <sup>b</sup>

<sup>a</sup>P < 0.05 compared with control mice.

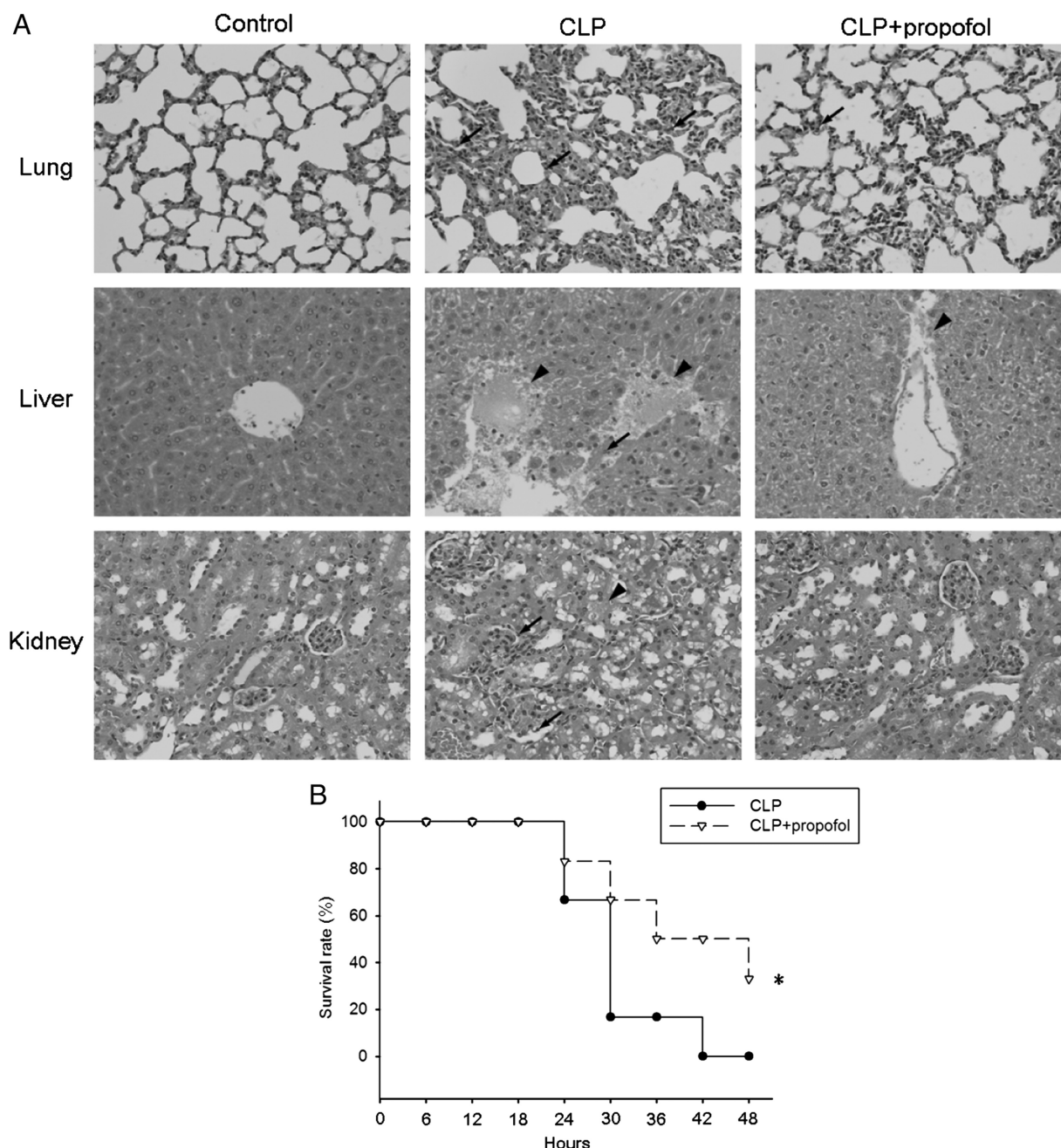
<sup>b</sup>P < 0.05 compared with CLP mice. Control: normal mice, CLP: mice received CLP surgery combined with saline treatment, CLP + propofol: mice received CLP surgery combined with propofol treatment. Sham + propofol: mice received sham surgery combined with propofol treatment. Values (mean  $\pm$  SE) were obtained from each group of six mice.

B55:5; Sigma-Aldrich Inc., USA) in combination with different concentrations of propofol (Sigma-Aldrich Inc., USA) for 24-h incubation. M1 cells were incubated in the normal medium or the propofol-containing medium for 5 h after 1-h  $H_2O_2$  (100  $\mu M$ ) exposure.

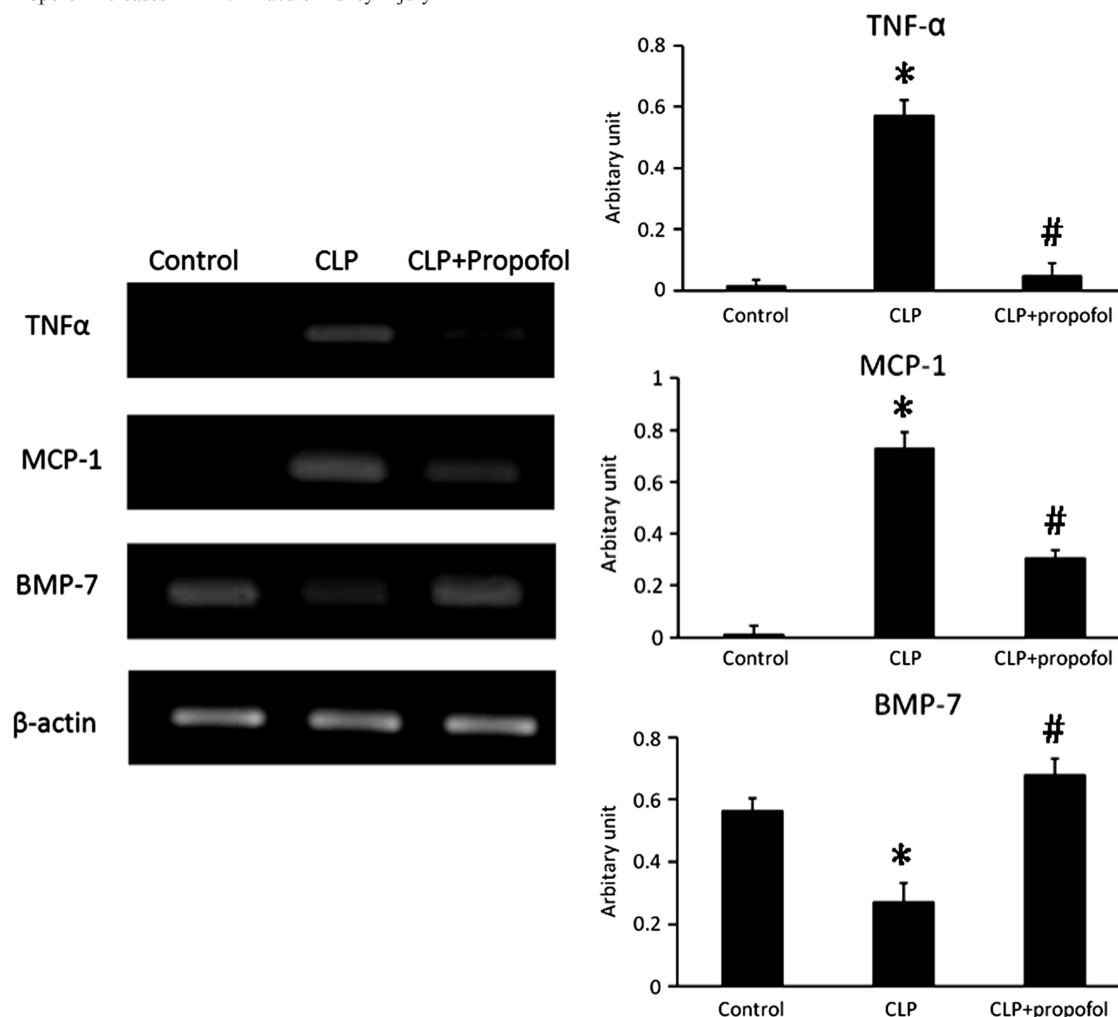
#### Reverse transcription–polymerase chain reaction

Reverse transcription–polymerase chain reaction (RT–PCR) was performed to determine the BMP-7, TNF- $\alpha$  or monocyte chemoattractant protein (MCP)-1 mRNA expression. Total RNA of kidney or renal cells was extracted using Trizol solution (Life Technologies) and subjected

to reverse transcription with StrataScript H-reverse transcriptase (Stratagene, La Jolla, CA) to generate cDNA. Gene-specific primer pairs (sense and antisense, respectively) used are as follows: BMP-7, F5'-CTGGATGGGCAGAGCATCAA-3' and R5'-TGTTGGTGGCGTTCATGTA-3'; TNF- $\alpha$ , F5'-GAGTGACAAGCCTGTAGCCCA-3' and R5'-CCCTTCTCAGCTGG AAGA-3'; MCP-1, F5'-ACCTGCTGTACTCATTAC-3' and R5'-TACAGAAGTGCTTGAGGTGG-3';  $\beta$ -actin, F5'-GCTGGAAGGTGGACAGCGAG-3' and R5'-TGGCATCGTGATGACTCCG-3'. PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide.  $\beta$ -Actin amplification was used as an internal control.



**Fig. 1.** Effects of propofol treatment on histological changes and survival of CLP mice. **(A)** Histological observation using H&E stain in the lung, liver and kidney of CLP mice. Arrow: neutrophil infiltration. Arrow head: necrotic region. **(B)** Kaplan–Meier survival curves of CLP mice treated with saline or propofol. \* $P < 0.05$  values of CLP mice compared with the values of CLP + propofol mice. Control: normal mice, CLP: mice received CLP surgery combined with saline treatment, CLP + propofol: mice received CLP surgery combined with propofol treatment.



**Fig. 2.** Effect of propofol on TNF- $\alpha$ , MCP-1 or BMP-7 expression in the kidney of CLP mice. The mRNA expression of TNF- $\alpha$ , MCP-1 or BMP-7 in the kidney of control, CLP and CLP + propofol mice ( $n = 6$  in each group) was detected using RT-PCR.  $\beta$ -Actin mRNA expression is internal control. Semi-quantification of mRNA levels is indicated as the mean  $\pm$  SE. \* $P < 0.05$  compared with control mice, # $P < 0.05$  compared with CLP mice. Control: normal mice, CLP: mice received CLP surgery combined with saline treatment, CLP + propofol: mice received CLP surgery combined with propofol treatment.

#### Intracellular ROS detection

About 5000 RMCs were seeded onto 12-well cell culture plates. Cultures were placed in control, LPS and/or propofol-containing medium for 24 h and then harvested. After 30-min fixation with 3.7% paraformaldehyde, cells were washed with phosphate-buffered saline (PBS) and then stained with 5  $\mu$ M dihydroethidium (DHE; Invitrogen). Images were collected with an Olympus IX70 fluorescence microscope.

#### Lucigenin assay for superoxide measurement

About 10 000 RMCs or M1 cells were incubated in control, LPS and/or propofol-containing medium for 1 h. Cells were then trypsinized, collected by centrifugation and washed. The pellet was then re-suspended in 900  $\mu$ L of Krebs buffer containing NaCl (130 mmol), KCl (5 mmol),  $MgCl_2$  (1 mmol),  $CaCl_2$  (1.5 mmol),  $K_2HPO_4$  (1 mmol) and HEPES (20 mmol, pH 7.4), with 1 mg/mL bovine serum albumin and then transferred into a measuring chamber. Suspensions were injected with 100  $\mu$ L lucigenin (final concentration,  $4 \times 10^{-4}$  mmol/L), and photon emissions were counted every 10 s for up to 3 min assessed by a chemiluminescence analyser (Tohoku Electronic Industrial Co., Ltd., Japan).

#### Determination of NO

The amount of nitrite in the culture medium of RMCs was detected by Measure-iT<sup>TM</sup> High-Sensitivity Nitrite Assay Kit (Molecular Probes,

Invitrogen Detection Technologies, USA) according to the technical bulletin.

#### MTT assay

RMCs were seeded in 96-well plates at a density of 10 000 cells/well. After 12 h, cells were treated with 0, 10, 50, 100 or 200  $\mu$ M propofol for 24 h in the presence of 0 or 100 ng/mL LPS. Then RMCs were washed three times with PBS. In the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma, USA] solution, 500  $\mu$ g/mL was pipetted into each well for 3-h incubation. After melting formazan made by adding 200  $\mu$ L dimethyl sulfoxide solution (Sigma, USA), absorbance was measured at a wavelength of 570 nm using a microplate reader (Bio-Rad Model 550; Hercules, CA, USA). Eight independent experiments were done.

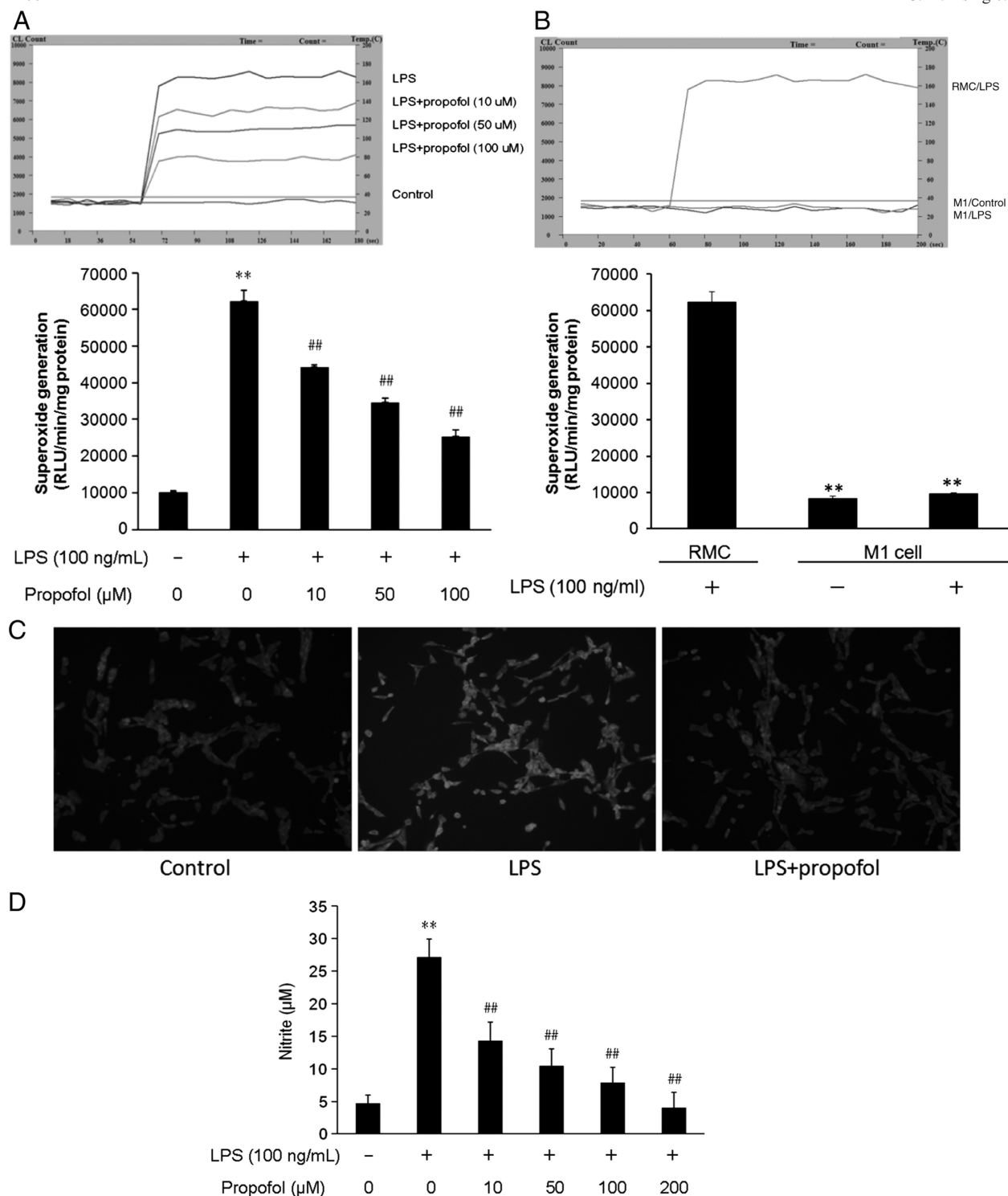
#### Cell death detection

The necrotic and apoptotic cells were observed after Hoechst 33342 Staining. After LPS or  $H_2O_2$  with or without propofol treatment, RMCs or M1 cells were fixed and stained with Hoechst 33342 (final concentration 0.002% of PBS) for 30 min. We observed the cells using Olympus IX70 fluorescence microscope.

#### PPAR- $\gamma$ and NF- $\kappa$ B activation analyses

PPAR- $\gamma$  and NF- $\kappa$ B in RMCs were detected by western blot analysis. Nuclear extracts were prepared using the nuclear extraction kit (Pa-

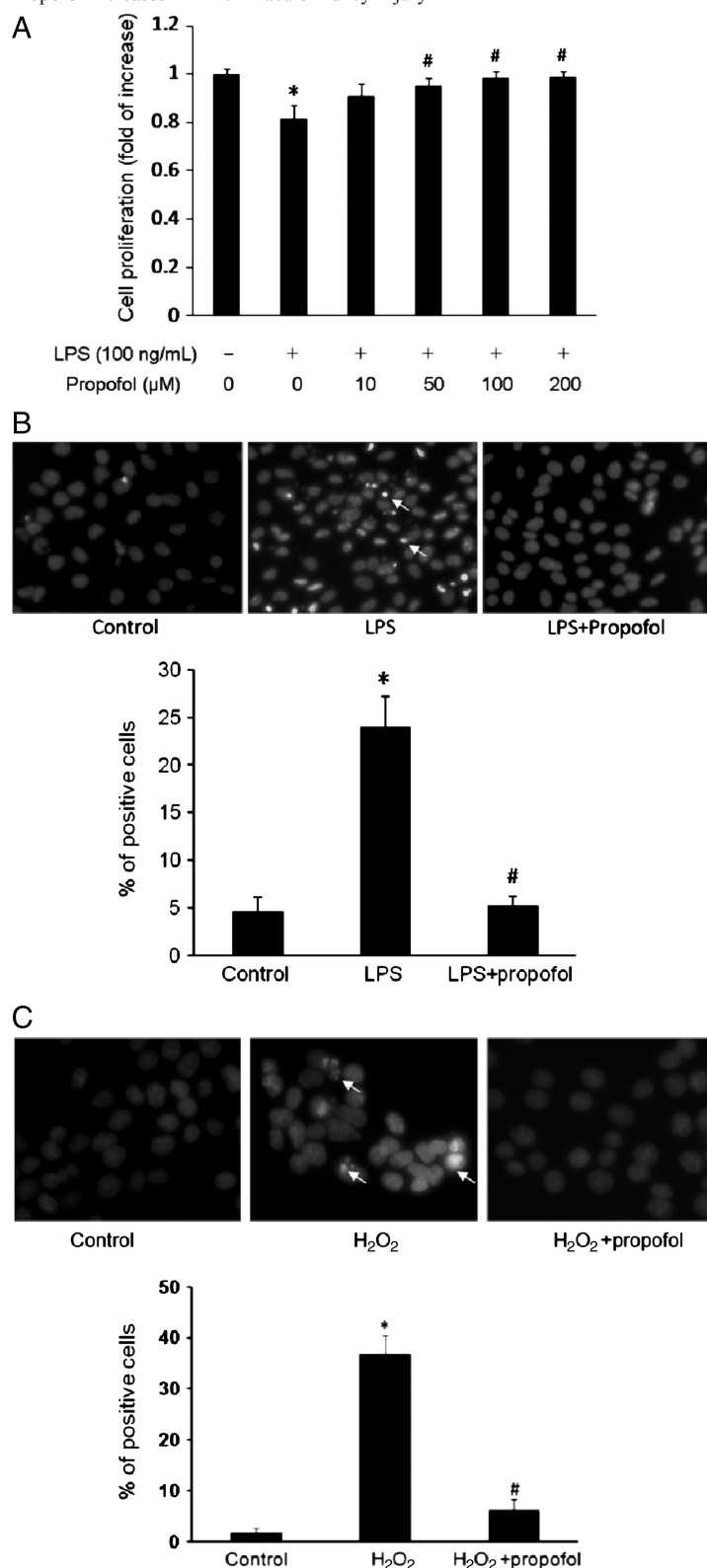




**Fig. 3.** Effect of propofol on free radicals generation in renal cells. (A) RMCs were cultured in normal medium or stimulated with LPS (100 ng/mL) in the presence of 10, 50 or 100  $\mu$ M propofol for 1 h. (B) M1 cells were cultured in normal medium or stimulated with LPS (100 ng/mL). RMCs stimulated with LPS (100 ng/mL) were taken as positive control. Superoxide generation in RMCs or M1 cells was detected using lucigenin assays. Representative curves in chemiluminescence analyser are shown in upper panel. Quantifications of the superoxide generation are shown in lower panel. (C) RMCs were cultured in normal medium or stimulated with LPS (100 ng/mL) or LPS plus propofol (50  $\mu$ M) for 24 h. ROS generation was observed using DHE staining. (D) Propofol (10, 50, 100 or 200  $\mu$ M) inhibited NO formation stimulated by LPS (100 ng/mL) in supernatant from RMCs using the Griess reaction. Data are presented as mean  $\pm$  SE ( $n = 8$ ). \*\* $P < 0.001$  compared with control, ## $P < 0.001$  compared with LPS.

nomics Inc., USA) and then separated by SDS-PAGE, transferred and immobilized on a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked by incubation with 5% non-fat dry milk in

PBS for 2 h. The membrane was then hybridized with PPAR- $\gamma$  or actin antibodies (1:1000; Santa Cruz, CA, USA) or NF- $\kappa$ B antibody (1:2000; Cell Signaling, CA, USA) diluted in PBS for 16 h. Incubation with



**Fig. 4.** Effect of propofol on cell viability of renal cells. (A) The viability of RMCs treated with different concentrations of propofol in the presence LPS or not was determined by MTT assay. Data represent mean  $\pm$  SE ( $n = 8$ ). (B) Cell death in RMCs was detected using Hoechst 33342 staining. Control: RMCs cultured in normal medium. LPS: RMCs were stimulated with LPS (100 ng/mL) for 24 h. LPS + propofol: LPS (100 ng/mL)-stimulated RMCs were treated with propofol (50  $\mu$ M) for 24 h. (C) M1 cell death was detected using Hoechst 33342 staining. Control: M1 cells cultured in normal medium for 6 h. H<sub>2</sub>O<sub>2</sub>: M1 cells were treated with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) for 1 h and then incubated in normal medium for further 5 h. H<sub>2</sub>O<sub>2</sub> + propofol: M1 cells were treated with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) for 1 h and then the culture medium was substituted with the medium containing propofol (50  $\mu$ M) for further 5-h incubation. The arrow indicates necrotic or apoptotic cells. The counts of cell death in RMCs or M1 cells are shown in the lower panel of (B) and (C). Data are means  $\pm$  SE ( $n = 3$ ). \* $P < 0.05$  compared with control, # $P < 0.05$  compared with LPS.

antibodies and detection of the antigen–antibody complex were performed using the ECL kit (Amersham, UK).

### Statistics

Results were expressed as mean  $\pm$  standard error (SE). We used Kaplan–Meier analysis to determine the survival distributions, and the logrank test was used to compare two survival distributions. The semi-quantification of RT–PCR densities and western blot results was quantified using ImageJ. Statistical analysis was performed using ANOVA analysis and Newman–Keuls post-hoc analysis. Statistical significance was set at  $P < 0.05$ .

## Results

### *Propofol reduced organ injuries and increased survival rate of CLP mice*

We used CLP mice model to establish polymicrobial sepsis-induced AKI. Twenty-four hours after CLP surgery, the mice showed characteristics of sepsis, including lethargy, piloerection, diarrhoea, huddling and malaise. The serum levels of CRP, GOT, GPT, BUN and creatinine were higher in CLP mice than in control mice ( $P < 0.05$ ) while propofol (50 mg/kg i.p.) treatment decreased those levels in CLP mice (Table 1). The serum levels of CRP, GOT, GPT, BUN and creatinine of propofol-treated sham-operated mice were not significantly different from those of control mice. In histological observations, CLP surgery induced neutrophil infiltration or sequestration in the kidney, lung and liver tissues (Figure 1A). In the liver, CLP caused wide-spread necrosis and centrilobular vacuolation of hepatocytes. In the kidney, CLP surgery induced vacuolar degeneration in the renal tubular epithelial cells and occasional neutrophil infiltration around glomeruli and in the interstitium. Propofol treatment inhibited neutrophil infiltration in the kidney, lung and liver and decreased the necrosis in the kidney and liver of CLP mice (Figure 1A). Propofol treatment also increased the survival of CLP mice (Figure 1B) 48 h after surgery.

### *Propofol decreased pro-inflammatory cytokine and increased BMP-7 expression in the kidney of CLP mice*

The TNF- $\alpha$  and MCP-1 mRNA expressions were higher but the BMP-7 mRNA expression was lower in the kidney of CLP mice when compared with those of the control mice (Figure 2). Propofol treatment decreased the TNF- $\alpha$  and MCP-1 mRNA expressions while it increased the BMP-7 mRNA expression in the kidney of CLP mice (Figure 2).

### *Propofol decreased oxidative stress in LPS-stimulated mesangial cells*

Propofol has been reported to be an antioxidant [27,28]. The ability of propofol to decrease LPS-stimulated superoxide generation in renal cells was measured by lucigenin assay. The superoxide generation in LPS-stimulated RMCs exhibited a 6-fold increase as compared with the control RMCs (Figure 3A). In contrast, LPS

did not stimulate detectable superoxide generation in M1 cells in this experiment (Figure 3B). Propofol treatment decreased LPS-induced superoxide generation in RMCs in a dose-dependent manner (Figure 3A). In DHE staining, LPS induced ROS generation in RMCs, but propofol treatment, a clinically relevant concentration (50  $\mu$ M) [1,36], could inhibit this (Figure 3C). NO production within glomeruli initiates chemical and cellular responses mediating inflammatory signals during sepsis-induced AKI [4]. We detected that the NO production in RMCs was increased after LPS stimulation. Propofol treatment dose-dependently decreased NO production in LPS-stimulated RMCs (Figure 3D).

### *Propofol decreased renal cell death*

LPS-induced cell death is critical in the pathogenesis of AKI during sepsis [9,10]. MTT assay showed that propofol treatment ameliorated the decreased viability in LPS-stimulated RMCs (Figure 4A). Hoechst 33342 staining was used to observe the LPS-induced cell death of RMCs. The number of necrotic and apoptotic cells increased, and propofol treatment decreased cell death in LPS-stimulated RMCs (Figure 4B). ROS produced by mesangial cells during sepsis, attacks renal tubular cells thus leading to kidney injury [2,3]. M1 cells were exposed to H<sub>2</sub>O<sub>2</sub> and the effect of propofol on cell death in these cells was observed. Propofol treatment prevented H<sub>2</sub>O<sub>2</sub>-induced M1 cell death (Figure 4C).

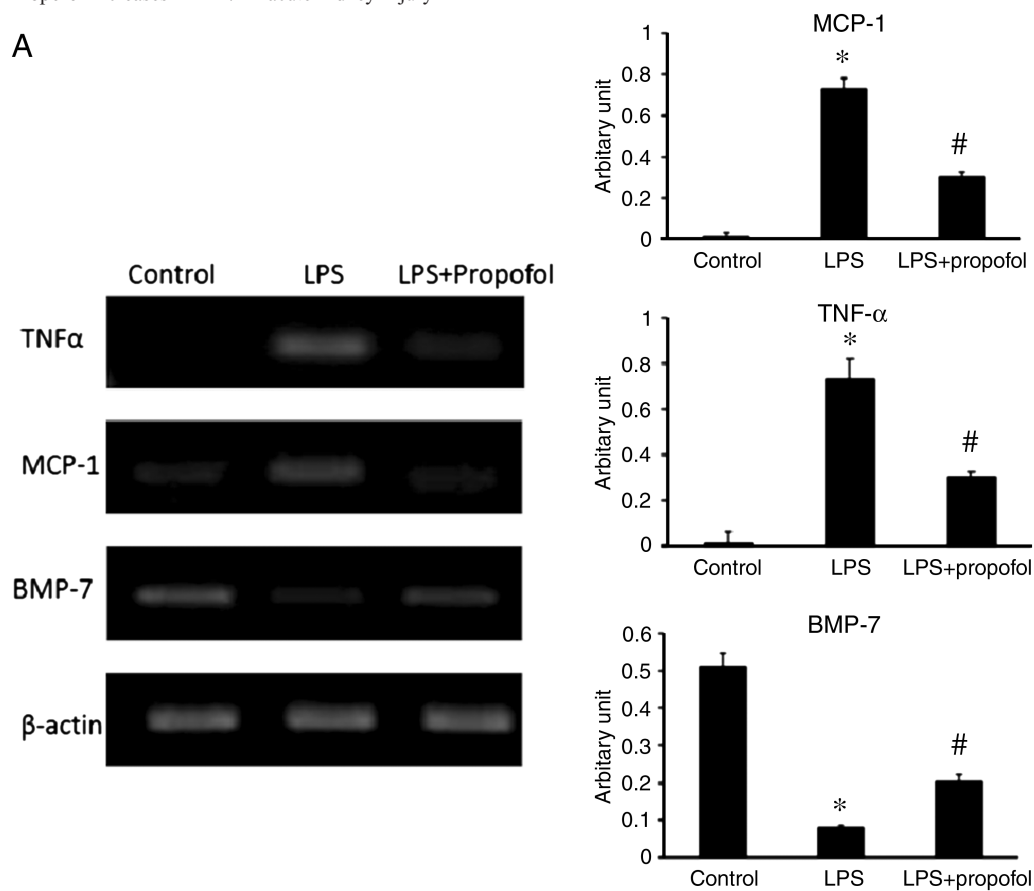
### *Propofol decreased inflammatory cytokines and increased BMP-7 mRNA expression in renal cells*

The mRNA of TNF- $\alpha$  and MCP-1 expressions was increased, while BMP-7 expression was decreased after 24-h LPS stimulation in RMCs (Figure 5). Propofol treatment decreased TNF- $\alpha$  and MCP-1 expressions while increased BMP-7 expression in LPS-stimulated RMCs (Figure 5). In M1 cells, H<sub>2</sub>O<sub>2</sub> exposure increased TNF- $\alpha$  expression but propofol treatment significantly decreased it (Figure 5B).

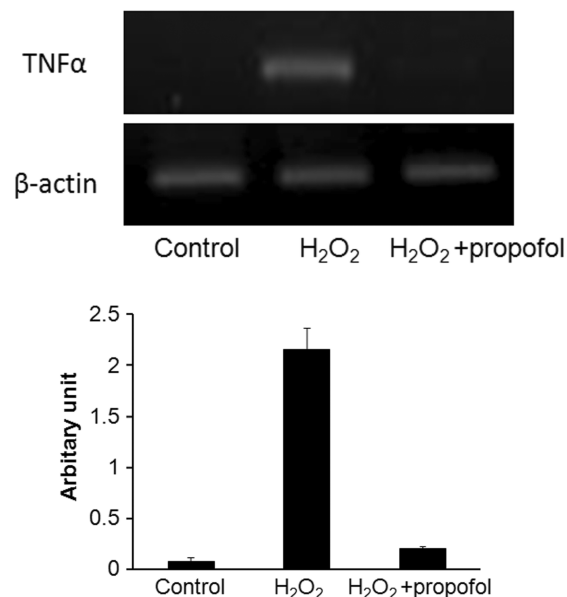
### *Propofol decreased NF- $\kappa$ B while increasing PPAR- $\gamma$ in nuclear extracts of LPS-stimulated mesangial cells*

TNF- $\alpha$  is a major inflammatory cytokine that activates the NF- $\kappa$ B signalling pathway in AKI [2]. PPAR- $\gamma$  agonists may reduce the serum level of TNF- $\alpha$  during sepsis by inhibiting NF- $\kappa$ B [14,15]. BMP-7 could attenuate the activity of NF- $\kappa$ B but increase the PPAR- $\gamma$  activity [21]. In this study, propofol treatment decreased TNF- $\alpha$  and increased BMP-7 expression in LPS-stimulated RMCs. Therefore, we hypothesized that propofol inhibits inflammation by regulating PPAR- $\gamma$  and NF- $\kappa$ B activation. To test this possibility, the activities of these two transcription factors in LPS-stimulated RMCs were analysed using western blot. To dissect the PPAR- $\gamma$  and NF- $\kappa$ B activation, the nuclear protein fractions were extracted as described in Materials and methods. The activity of PPAR- $\gamma$  was inhibited (Figure 6A) but the activity of NF- $\kappa$ B was in-

A

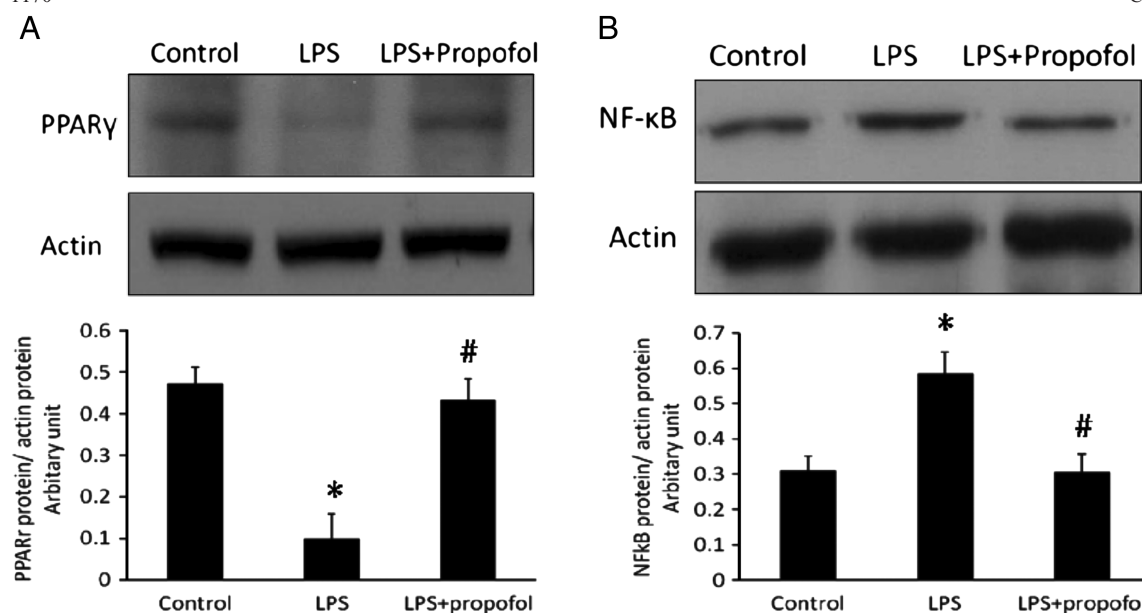


B



**Fig. 5.** Effect of propofol on pro-inflammatory cytokine expression in LPS-stimulated RMCs or H<sub>2</sub>O<sub>2</sub>-treated M1 cells. **(A)** TNF- $\alpha$ , MCP-1 or BMP-7 mRNA expression in LPS-stimulated RMCs was detected using RT-PCR. Control: RMCs were cultured in normal medium. LPS: RMCs were stimulated with LPS (100 ng/mL) for 24 h. LPS + propofol: LPS (100 ng/mL)-stimulated RMCs were treated with propofol (50  $\mu$ M) for 24 h. **(B)** TNF- $\alpha$  mRNA expression in H<sub>2</sub>O<sub>2</sub>-treated M1 cells. Control: M1 cells cultured in normal medium for 6 h. H<sub>2</sub>O<sub>2</sub>: M1 cells were treated with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) for 1 h and then incubated in normal medium for further 5 h. H<sub>2</sub>O<sub>2</sub> + propofol: M1 cells were treated with H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M) for 1 h and then the culture medium was substituted with the medium containing propofol (50  $\mu$ M) for further 5-h incubation.  $\beta$ -Actin mRNA expressions were internal control. Semi-quantification of mRNA levels is indicated as the mean  $\pm$  SE ( $n = 3$ ). \* $P < 0.05$  compared with control, # $P < 0.05$  compared with LPS.





**Fig. 6.** Effect of propofol on PPAR- $\gamma$  and NF- $\kappa$ B activation in LPS-stimulated RMCs. PPAR- $\gamma$  (A) and NF- $\kappa$ B (B) expressions in nuclear extracts of RMCs were determined using western blots. Control: RMCs were cultured in normal medium. LPS: RMCs were stimulated with LPS (100 ng/mL) for 24 h. LPS + propofol: LPS (100 ng/mL)-stimulated RMCs were treated with propofol (50  $\mu$ M) for 24 h. The upper panel shows the protein level of PPAR- $\gamma$ , NF- $\kappa$ B and actin in RMCs. Semi-quantification of protein levels is indicated as the mean  $\pm$  SE ( $n = 3$ ) in each column and is presented in the lower panel. \* $P < 0.05$  compared with control, # $P < 0.05$  compared with LPS.

creased (Figure 6B) after LPS stimulation in RMCs. The decreased levels of PPAR- $\gamma$  activity and the increased levels of NF- $\kappa$ B activity in LPS-stimulated RMCs were reversed to the control level by propofol treatment (Figure 6A and B).

## Discussion

The present study performed CLP surgery in mice, which causes lethal peritonitis by microbial infection and is a valid animal model for human sepsis [29]. Consistent with previous reports [27–29], propofol treatment significantly reduced mortality in murine model of sepsis. This study also demonstrates that propofol treatment could ameliorate sepsis-induced AKI through inhibiting oxidative stress, decreasing inflammatory cytokine expression and increasing BMP-7 expression. In addition to the renal protective effect, the serum biochemical profiles and pathological observations in CLP mice implied that propofol treatment could decrease lung and liver injury in CLP mice. Although the mortality of CLP mice was decreased by propofol treatment, the obvious liver injury remained in propofol-treated CLP mice. The molecular mechanism of extra-renal effects of propofol on reducing mortality and organ injury in sepsis needs further investigations.

TNF- $\alpha$  or MCP-1 recruits neutrophil infiltrating into the renal tissue and amplifies acute tubular injury during sepsis-induced AKI [8,11,12]. Propofol was used for anti-inflammation by inhibiting cytokine and chemokine production [29]. The results showed that propofol decreased TNF- $\alpha$  and MCP-1 expressions in the kidney of

CLP mice, LPS-stimulated RMCs and H<sub>2</sub>O<sub>2</sub> exposed M1 cells. Propofol treatment also decreased the neutrophil infiltration in glomeruli and interstitium in pathological observations. These results imply that propofol could decrease neutrophil infiltration in kidney by decreasing TNF- $\alpha$  and MCP-1 during sepsis.

Free radical generation is increased in multiple organs during sepsis [33,37,38]. Oxidative stress in sepsis and the link with inflammatory gene expression are foundations for intervention by either reducing oxidative stress or inhibiting transcriptional activation of inflammatory cytokines using antioxidants [3,38,39]. TNF- $\alpha$  induces mesangial and endothelial cells to produce ROS and NO [13]. Pentoxifylline, could protect against endotoxaemia-related AKI by decreasing serum TNF- $\alpha$  and NO [40,41]. Propofol, containing a phenol hydroxyl group, conferring antioxidant activity, could inhibit inflammatory response by decreasing free radical production [25,26,30]. In this study, propofol inhibited the LPS-induced ROS and NO generation in RMCs dose dependently and decreased LPS-induced cell death. In addition to mesangial cells, the protective effect of propofol on renal tubular cells was examined since they are susceptible by oxidative stress during sepsis-induced AKI [2,4]. A previous study indicated that renal tubules produced ROS on stimulation by phorbol 12-myristate 13-acetate (PMA), but the response was relatively minor compared with that of glomeruli [42]. In the present study, LPS could stimulate ROS generation in mesangial cells but not in tubular epithelial cells (Figure 3A and B). Tubular cells may not be the main superoxide producing cells but ROS can damage tubular cells during sepsis [3–5]. The present study also showed that propofol could reduce H<sub>2</sub>O<sub>2</sub>-induced oxidative stress injury in renal

tubular cells. These results suggest that propofol not only inhibits free radical generation in mesangial cells but also decreases oxidative stress-induced injury in the kidney.

BMP-7 plays a protective role in maintaining the structure and function of podocytes, mesangial cells and distal tubular or collecting duct epithelial cells [43]. However, BMP-7 expression disappears early by oxidative stress suppression in acute or chronic renal diseases [16–19,44]. The change of BMP-7 expression in sepsis-induced AKI has not been reported. This study revealed that renal BMP-7 mRNA expression was decreased in the kidneys of CLP mice and in LPS-stimulated RMCs, which suggest that BMP-7 plays a role in sepsis-induced AKI. Elevating BMP-7 could be a therapeutic strategy for kidney disease [44]. Whether BMP-7 is indeed directly related to propofol could be examined on BMP-7-deficient mice in a further study. Antioxidant treatment may increase renal BMP-7 and protect renal function in rat diabetic nephropathy [22,45]. BMP-7 has antioxidative ability against high glucose-induced oxidative stress in mesangial cells [23]. According to the *in vivo* observations, propofol treatment decreased the serum level of BUN and creatinine as well as reduced kidney injury in CLP mice. Propofol treatment also increased BMP-7 expression in the kidney of CLP-induced AKI mice. *In vitro*, propofol treatment depressed oxidative stress while increased BMP-7 expression in renal cells. These results suggest that propofol could increase renal BMP-7 expression to protect mice from CLP-induced AKI by decreasing oxidative stress during sepsis.

During sepsis, activation of NF- $\kappa$ B leads to an increase in cytokines and chemokines expression which causes organ injury. Blocking NF- $\kappa$ B could be a strategy to protect against endotoxaemia [2]. On the other hand, PPAR- $\gamma$  agonists may exert their anti-inflammatory effects by negatively regulating the expression of TNF- $\alpha$  through inhibition of NF- $\kappa$ B [14,15]. BMP-7 could attenuate NF- $\kappa$ B nuclear translocation stimulated by TNF- $\alpha$  in pIgA-induced nephropathy and this anti-inflammatory effect of BMP-7 is associated with PPAR- $\gamma$  activation [21]. This paper shows that propofol could decrease LPS-induced TNF- $\alpha$  expression and NF- $\kappa$ B activation in renal cells. Furthermore, propofol could increase the expressions of the renal protective protein, BMP-7, and the activity of anti-inflammatory factor, PPAR- $\gamma$ . These results suggest that propofol could decrease inflammatory responses by increasing BMP-7 expression and the activity of PPAR- $\gamma$  to depress TNF- $\alpha$ /NF- $\kappa$ B axis in LPS-stimulated RMCs.

## Conclusion

In conclusion, propofol could decrease inflammatory cytokine and oxidative stress by inhibiting free radical generation and increasing BMP-7 expression during sepsis-induced AKI. Propofol could also regulate the factors responsible for inflammation, including decreasing TNF- $\alpha$ -mediated NF- $\kappa$ B pathway and increasing PPAR- $\gamma$  expression. This research provides a possible explanation to the molecular mechanism of propofol in protecting

kidney from sepsis-induced AKI. Thus, the widely used sedative agent propofol might have therapeutic potential in reducing AKI in sepsis.

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**Conflict of interest statement.** None declared.

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