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Vitamin D3 pretreatment alleviates renal oxidative stress in lipopolysaccharide-induced acute kidney injury

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ABSTRACT

Increasing evidence demonstrates that reactive oxygen species plays important roles in sepsis-induced acute kidney injury. This study investigated the effects of VitD3 pretreatment on renal oxidative stress in sepsis-induced acute kidney injury. Mice were intraperitoneally injected with lipopolysaccharide (LPS, 2.0 mg/kg) to establish an animal model of sepsis-induced acute kidney injury. In VitD3 + LPS group, mice were orally pretreated with three doses of VitD3 (25 μg/kg) at 1, 24 and 48 h before LPS injection. As expected, oral pretreatment with three daily recommended doses of VitD3 markedly elevated serum 25 (OH)D concentration and efficiently activated renal VDR signaling. Interestingly, LPS-induced renal GSH depletion and lipid peroxidation were markedly alleviated in VitD3-pretreated mice. LPS-induced serum and renal nitric oxide (NO) production was obviously suppressed by VitD3 pretreatment. In addition, LPS-induced renal protein nitration, as determined by 3-nitrotyrosine residue, was obviously attenuated by VitD3 pretreatment. Further analysis showed that LPS-induced up-regulation of renal inducible nitric oxide synthase (*inos*) was repressed in VitD3-pretreated mice. LPS-induced up-regulation of renal *p47phox* and *gp91phox*, two NADPH oxidase subunits, were normalized by VitD3 pretreatment. In addition, LPS-induced down-regulation of renal *superoxide dismutase (sod) 1* and *sod2*, two antioxidant enzyme genes, was reversed in VitD3-pretreated mice. Finally, LPS-induced tubular epithelial cell apoptosis, as determined by TUNEL, was alleviated by VitD3 pretreatment. Taken together, these results suggest that VitD3 pretreatment alleviates LPS-induced renal oxidative stress through regulating oxidant and antioxidant enzyme genes.

1. Introduction

Acute kidney injury, defined as a rapid renal dysfunction with severe tubular damage, is a frequent and serious complication of sepsis in intensive care unit (ICU) patients with an extremely high mortality [1–3]. It is increasingly recognized that sepsis is the most important causes of acute kidney injury in critically ill patients, account for 50% or more of cases of acute kidney injury in ICUs [4,5]. Lipopolysaccharide (LPS) is a component of the outer membrane in Gram-negative bacteria and humans are constantly exposed to low levels of LPS through infection [6]. LPS is widely present in the digestive tracts of humans and animals and gastrointestinal inflammatory diseases and excess alcohol intake are known to increase permeability of LPS from gastrointestinal tract into blood [7]. Increasing evidence demonstrates that LPS is involved in the pathogenesis of sepsis-induced acute kidney injury [8,9]. Thus, LPS has been widely used as a model of experimental sepsis-induced acute kidney injury [10]. As there has been no effective treatment for sepsis-induced acute kidney injury, novel preventive and therapeutic interventions are urgently needed to tackle sepsis-induced acute kidney injury.

Numerous reports demonstrate that reactive oxygen species (ROS) plays an important role in sepsis-induced acute kidney injury [11–14]. In addition, reactive nitrogen species (RNS) is involved in sepsis-induced acute kidney injury [15,16]. Several

Abbreviations: LPS, lipopolysaccharide; VitD3, vitamin D3; NO, nitric oxide; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; inos, inducible nitric oxide synthase; BUN, blood urea nitrogen.

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studies showed that antioxidants alleviated sepsis-induced acute kidney injury [17,18]. Vitamin D is known for its classical functions in calcium uptake and bone metabolism [19]. Recently, vitamin D is recognized for its non-classical actions including modulation of innate immune response, regulation of cell proliferation and improvement of hippocampal synaptic function [20–22]. Several studies showed that vitamin D had an anti-oxidant activity [23,24]. Thus, it is especially interesting whether vitamin D supplementation alleviates renal oxidative stress in sepsis-induced acute kidney injury.

In the present study, we investigated the effects of vitamin D3 (VitD3) on renal oxidative stress during LPS-induced acute kidney injury. Our results showed that VitD3 pretreatment protected mice from LPS-induced acute kidney injury. We demonstrate for the first time that VitD3 pretreatment alleviates LPS-evoked renal oxidative stress through regulating renal oxidant and anti-oxidant enzyme genes.

2. Materials and methods

2.1. Chemicals and reagents

Escherichia coli LPS (serotype 0127:B8) and VitD3 were purchased from Sigma Chemical Co. (St., Louis, MO). 3-Nitrotyrosine (3-NT), VDR and Lamin A/C antibodies were from Santa Cruz Biotechnologies (Santa Cruz, CA). TRI reagent was from Molecular Research Center, Inc. (Cincinnati, Ohio). RNase-free DNase was from Promega Co. (Madison, WI). All other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals

Adult male CD-1 mice (8 week-old, 28–32 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and maintained on a 12 h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment. All animals were euthanized with carbon dioxide and cervical dislocation. This study was approved by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University (Permit Number: 14-0011). All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Animal model and experimental treatments

Ninety-six mice were divided into four groups randomly (twenty-four mice each group). In LPS group, mice were intraperitoneally (i.p.) injected with a single dose of LPS (2.0 mg/kg). In NS group, mice were i.p. injected with normal saline (NS). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) by gavage at 48, 24 and 1 h before LPS injection. In VitD3 group, mice were pretreated with three doses of VitD3 (25 μg/kg) by gavage at 48, 24 and 1 h before NS injection. The doses of VitD3 used in the present study referred to others [25]. All mice were euthanized with carbon dioxide and cervical dislocation. Mice were sacrificed at different time point (either 1 or 6 or 24 h) after LPS injection. Blood samples were collected for measurement of renal function. Left kidneys were collected for histopathology. Right kidneys were collected and kept at −80 °C for subsequent experiments.

2.4. Tissue preparation and biochemical analysis

Blood samples were collected 24 h after LPS injection. Serum uric acid and blood urea nitrogen (BUN) were measured with colorimetric detection kits. The level of urinary albumin was measured using enzyme-linked immunosorbent assay (ELISA). For preparation of renal homogenates, 100 mg of kidney tissue was homogenized on ice in 1 ml of homogenization buffer (50 mM Tris–HCl, 180 mM KCl, 10 mM EDTA, pH 7.4). Renal malondialdehyde (MDA) was determined according to others [26]. The levels of MDA were expressed as nmol/mg kidney. Renal GSH content was measured as described by others [27]. GSH contents were expressed as nmol/mg kidney. Sera were collected 6 h after LPS injection. Serum nitrate plus nitrite, the stable end products of L-arginine-dependent nitric oxide (NO) synthesis, was measured using a colorimetric method based on the Griess reaction [28].

2.5. Measurement of 25(OH)D

Blood samples were collected and stored at −80 °C, with no further freeze-thaw cycles, until 25(OH)D measurement. Serum 25 (OH)D was measured by radioimmunoassay (RIA) with 125 I labeled 25(OH)D as a tracer [29,30], using a kit from Diasorin (DiaSorin Inc., Stillwater, MN, USA) following manufacturer's instructions. Serum 25(OH)D is expressed as ng/ml.

2.6. Isolation of total RNA and real-time RT-PCR

Total RNA in renal tissue was extracted using TRI reagent. RNase-free DNase-treated total RNA (1.0 μg) was reverse-transcribed with AMV (Promega). Real-time RT-PCR was performed with a LightCycler 480 SYBR Green I kit (Roche Diagnostics GmbH) using gene-specific primers as listed in Table 1. The amplification reactions were carried out on a LightCycler 480 Instrument (Roche Diagnostics GmbH) with an initial hold step (95 °C for 5 min) and 50 cycles of a three-step PCR (95 °C for 15 s, 60 °C for 15 s, 72 °C for 30 s).

2.7. Immunohistochemistry

Renal tissues were fixed in 4% formaldehyde and embedded in paraffin according to the standard procedure. Paraffin-embedded

Table 1
Primers for real-time RT-PCR.

Gene	Sequence	Length
18S	Forward: 5′-GTAACCCGTTGAACCCCATT-3′ Reverse: 5′-CCATCCAATCGGTAGTAGCG-3′	151
inos	Forward: 5′-GCTCGCTTTGCCACGGACGA-3′ Reverse: 5′-AAGGCAGCGGGCACATGCAA-3′	146
p47phox	Forward: 5′-CCAGGGCACTCTCACTGAATA-3′ Reverse: 5′-ATCAGGCCGCACTTTGAAGAA-3	100
nox4	Forward: 5′-CCAAATGTTGGGCGATTGTGT-3′ Reverse: 5′-TCCTGCTAGGGACCTTCTGT-3′	133
gp91phox	Forward: 5′-GGGAACTGGGCTGTGAATGA-3′ Reverse: 5′-CAGTGCTGACCCAAGGAGTT-3′	147
p67phox	Forward: 5′-GCTGCGTGAACACTATCCTGG-3′ Reverse: 5′-AGGTCGTACTTCTCCATTCTGTA-3	136
sod1	Forward: 5′-GCGATGAAAGCGGTGTGCGTG-3′ Forward: 5′-TGGACGTGGAACCCATGCTGG-3′	143
sod2	Reverse: 5′-AGCGAACGGCCGTGTTCTGAG-3 Forward: 5′-AGCGCGCCATAGTCGTAAGGC-3′	162
catalase	Forward: 5′-CGCGCTCGAGTGGCCAACT-3′ Forward: 5′-TGCTGCTCTGGTGCGCTGAA-3′	107
gshpx	Forward: 5′-GGTGGTGCTCGGTTTCCCGT-3′ Forward: 5′-AATTGGGCTCGAACCCGCCAC-3′	113
gshrd	Reverse: 5′-GGGATGCCTATGTGAGCCGCC-3 Forward: 5′-TGACTTCCACCGTGGGCCGA-3′	107

renal tissues were serially sectioned. At least five consecutive longitudinal sections were stained with hematoxylin and eosin (H&E). For immunohistochemistry, paraffin-embedded renal sections were deparaffinized and rehydrated in a graded ethanol series. After antigen retrieval and quenching of endogenous peroxidase, sections were incubated with 3-NT monoclonal antibody (1:200 dilution) for 18 h at 4 °C. The color reaction was developed with HRP-linked polymer detection system and counterstaining with hematoxylin.

2.8. Terminal dUTP nick-end labeling (TUNEL) staining

For the detection of apoptosis, paraffin-embedded sections were stained with the TUNEL technique using an in situ apoptosis detection kit (Promega Madison, WI) according to the manufacturer's protocols. TUNEL-positive cells were counted in twelve randomly selected fields from each slide at a magnification of ×400. The percentage of TUNEL-positive cells was analyzed in six kidney sections from six different mice.

2.9. Statistical analysis

Normally distributed data were expressed as means ± SEM. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups. Data that were not normally distributed were assessed for significance using non-parametric tests techniques (Kruskal–Wallis test and Mann–Whitney *U* test). $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Effects of VitD3 pretreatment on renal function and histopathology during LPS-induced acute kidney injury

The effects of VitD3 pretreatment on LPS-induced renal histopathology were analyzed. As expected, a mild pathological damage in the renal cortex and outer medulla, as showed by tubular epithelial cells sloughing, tubular dilation and distortion, was observed 24 h after LPS injection. Interestingly, VitD3 pretreatment obviously attenuated LPS-induced pathological damage in the kidneys (Fig. 1A). The effects of VitD3 pretreatment on renal function during LPS-induced acute kidney injury were analyzed. As expected, the levels of serum uric acid and BUN were markedly increased 24 h after LPS injection (Fig. 1B and C). In addition, the level of urinary albumin was obviously increased 24 h after LPS injection (Fig. 1D). Interestingly, LPS-induced elevation of serum uric acid and BUN was significantly attenuated in VitD3-pretreated mice (Fig. 1B and C). Moreover, LPS-induced elevation of urinary albumin was obviously alleviated by VitD3 pretreatment (Fig. 1D).

3.2. Effects of VitD3 pretreatment on serum 25(OH)D concentration and nuclear VDR level in the kidneys

The effects of VitD3 pretreatment on serum 25(OH)D concentration were analyzed. As shown in Fig. 2A, serum 25(OH)D concentration was significantly elevated in VitD3-pretreated mice. To explore whether VitD3 pretreatment activates VDR signaling in the kidneys, nuclear VDR level in the kidneys was measured. As expected, nuclear VDR level in the kidneys was significantly elevated in VitD3-pretreated mice (Fig. 2B).

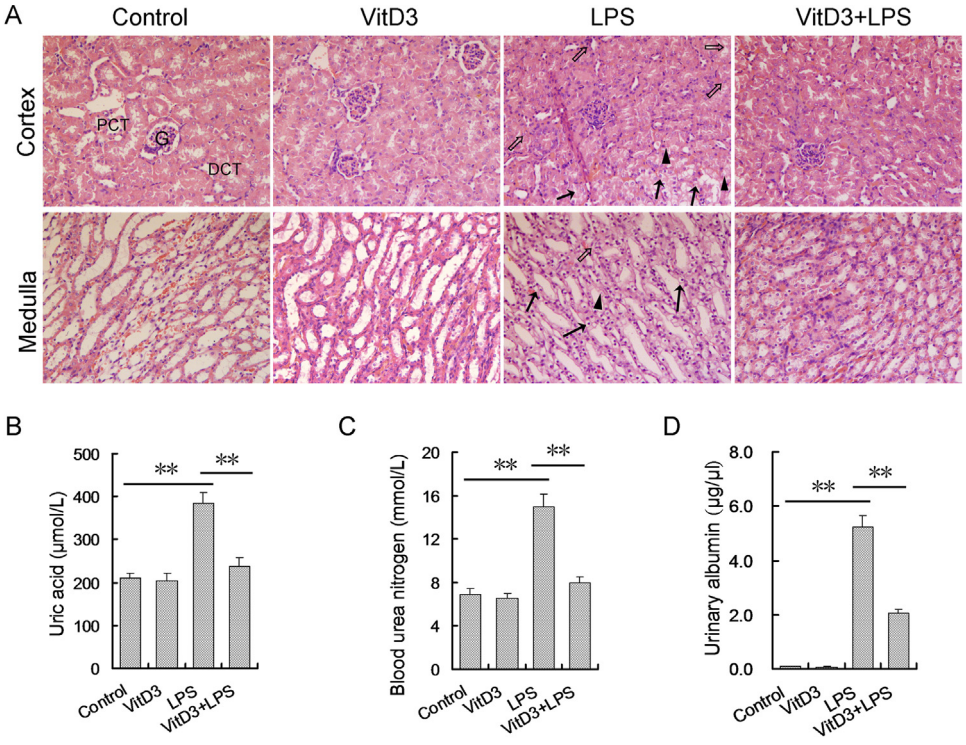

Fig. 1. VitD3 pretreatment alleviates on LPS-induced acute kidney injury. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidney and blood samples were collected 24 h after LPS injection. (A) Representative H&E staining of kidney cortex and outer medulla tissues. LPS-induced acute kidney injury with tubular dilation or cast formation (arrowheads), vacuolization of renal tubular cells (solid arrows) and the denuded tubular epithelial cells clump (hollow arrows). DCT: distal convoluted tubule; PCT: proximal convoluted tubule; G: glomerulus. Original magnification ×200. (B–D) Renal function was measured at 24 h after LPS injection. (B) Uric acid; (C) BUN; (D) Urinary albumin. All data were expressed as means ± SEM ($n = 10$). $^{**}P < 0.01$.

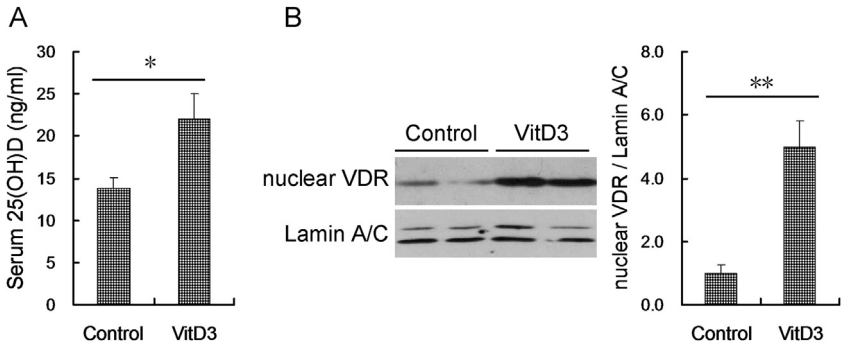

Fig. 2. Effects of VitD3 pretreatment on serum 25(OH)D concentration and nuclear VDR level in the kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidney and blood samples were collected 6 h after LPS injection. (A) Serum 25(OH)D level was measured by RIA. (B) Nuclear VDR was measured using immunoblots. All experiments were duplicated for three times. A representative gel for VDR (upper panel) and lamin A/C (lower panel) was shown. All data were expressed as means ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

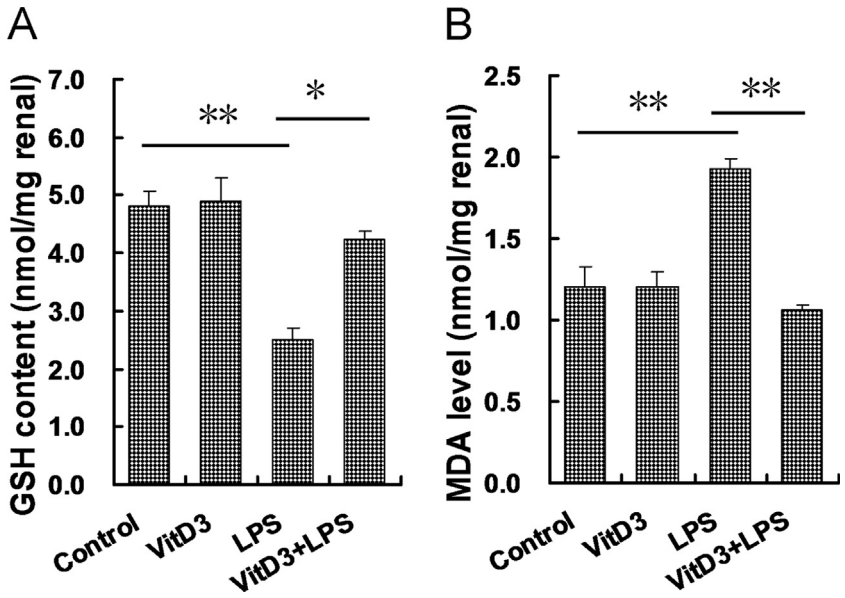

Fig. 3. Effects of VitD3 pretreatment on LPS-induced GSH depletion and lipid peroxidation in kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidney samples were collected 6 h after LPS injection. (A) Renal GSH content. (B) Renal MDA content. All data were expressed as means ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

3.3. VitD3 pretreatment alleviates LPS-induced renal GSH depletion and lipid peroxidation

The effects of VitD3 pretreatment on LPS-induced renal GSH depletion and lipid peroxidation were analyzed. As expected, renal GSH content was obviously decreased 6 h after LPS injection (Fig. 3A). In contrast, renal TBARS content, a marker of lipid peroxidation, was obviously increased 6 h after LPS injection (Fig. 3B). Interestingly, LPS-induced renal GSH depletion was markedly attenuated in VitD3-pretreated mice (Fig. 3A). In addition, LPS-induced renal lipid peroxidation was reversed by VitD3 pretreatment (Fig. 3B).

3.4. VitD3 pretreatment alleviates LPS-induced protein nitration in the kidneys

In order to indirectly infer the production of peroxynitrite, renal 3-NT residue, a marker of protein nitration, was evaluated 6 h after LPS injection. As shown in Fig. 4, strong 3-NT immunoreactivity was detected in the kidneys 6 h after LPS injection. Of interest, VitD3 pretreatment obviously alleviated LPS-induced protein nitration in the kidneys.

3.5. VitD3 pretreatment inhibits LPS-induced renal inos *expression and NO production*

To verify the effects of VitD3 pretreatment on LPS-induced NO production, the levels of serum and renal nitrite plus nitrate were measured 6 h after LPS injection. As expected, the levels of serum and renal nitrite plus nitrate were significantly increased 6 h after LPS injection (Fig. 5A and B). Interestingly, LPS-induced serum and renal NO productions were significantly attenuated in VitD3-pretreated mice. The effects of VitD3 pretreatment on LPS-induced upregulation of renal *inos* are shown in Fig. 5C. As expected, renal *inos* mRNA was significantly elevated 6 h after LPS injection. Interestingly, LPS-induced renal *inos* up-regulation was significantly attenuated in VitD3-pretreated mice.

3.6. VitD3 pretreatment inhibits LPS-induced NADPH oxidase subunits in the kidneys

As shown in Fig. 6A and C, renal *p47phox* and *gp91phox*, two NADPH oxidase subunits, were up-regulated 6 h after LPS injection. In contrast, renal *nox4* was markedly down-regulated 6 h after LPS injection (Fig. 6B). LPS had no effect on renal *p67phox* (Fig. 6D).

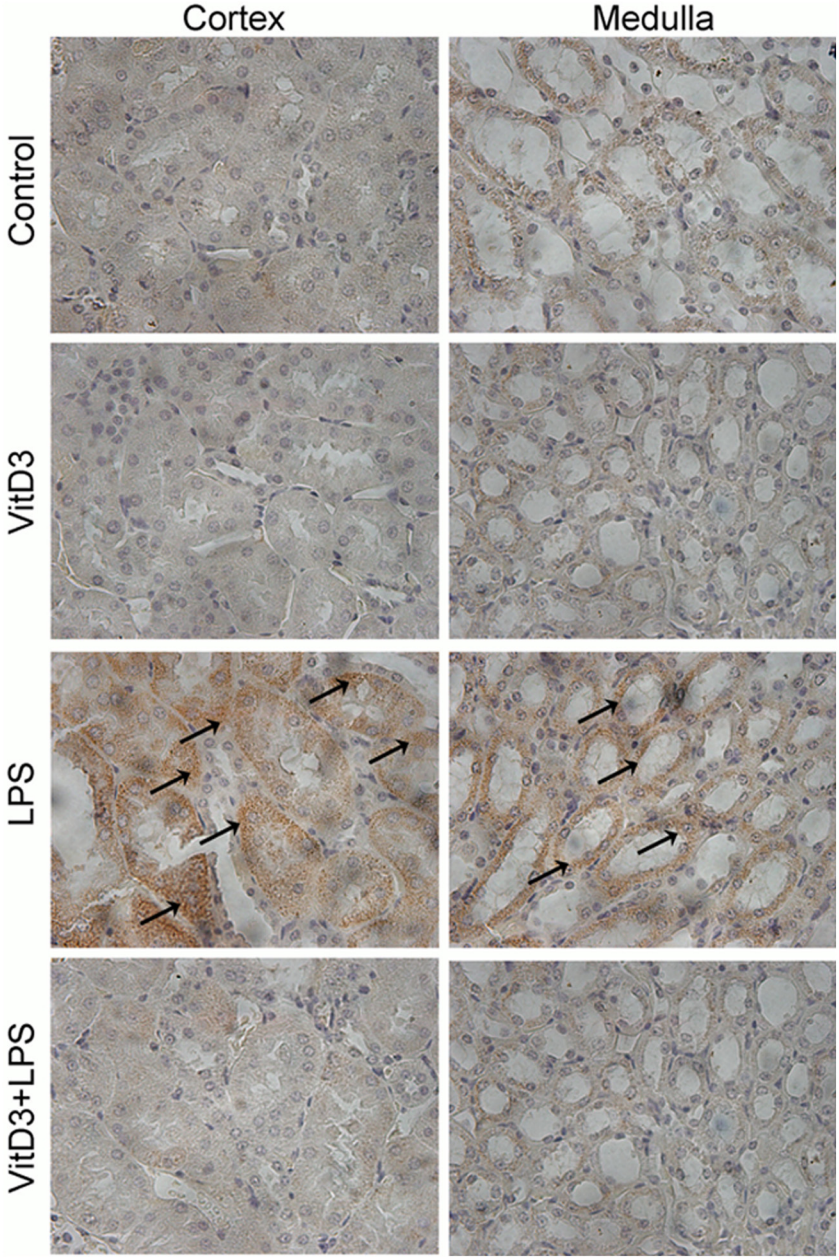

Fig. 4. Effects of VitD3 pretreatment on LPS-induced protein nitration in kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidneys were collected 6 h after LPS injection. Renal 3-NT residues were analyzed by immunohistochemistry. Representative photomicrographs of renal histological specimens from mice treated with NS, VitD3 alone, LPS alone and VitD3 + LPS are shown. Original magnification ×400. There was only minimal 3-NT immunoreactivity in kidneys of mice treated with salt or VitD3 alone. Renal 3-NT was mainly observed in tubular epithelial cells (arrows) of LPS-treated mice.

Interestingly, LPS-induced up-regulation of renal *p47phox* and *gp91phox* was significantly attenuated in VitD3-pretreated mice (Fig. 6A and C). However, VitD3 pretreatment had no effect on LPS-induced down-regulation of *nox4* in the kidneys (Fig. 6B).

3.7. VitD3 pretreatment attenuates LPS-induced down-regulation of antioxidant enzyme genes

As shown in Fig. 7A and B, renal *superoxide dismutase (sod) 1* and *sod2*, two antioxidant enzyme genes, were obviously down-regulated 1 h after LPS injection, whereas LPS had little effect on the expression of renal *catalase*, *glutathione peroxidase (gshpx)* and *glutathione reductase (gshrd)* (Fig. 7C–E). Interestingly, LPS-induced down-regulation of renal *sod1* and *sod2* was markedly attenuated in VitD3-pretreated mice (Fig. 7A and B).

3.8. VitD3 pretreatment inhibits LPS-induced cell apoptosis in the kidneys

The effects of VitD3 pretreatment on LPS-induced cell apoptosis in the kidneys were analyzed. As shown in Fig. 8, a number of $TUNEL^+$ cells were observed in cortex and medulla 6 h after LPS injection. Interestingly, LPS-induced tubular cell apoptosis in cortex was almost completely blocked in VitD3-pretreated mice. In addition, LPS-induced tubular cell apoptosis in medulla was markedly attenuated by VitD3 pretreatment (Fig. 8).

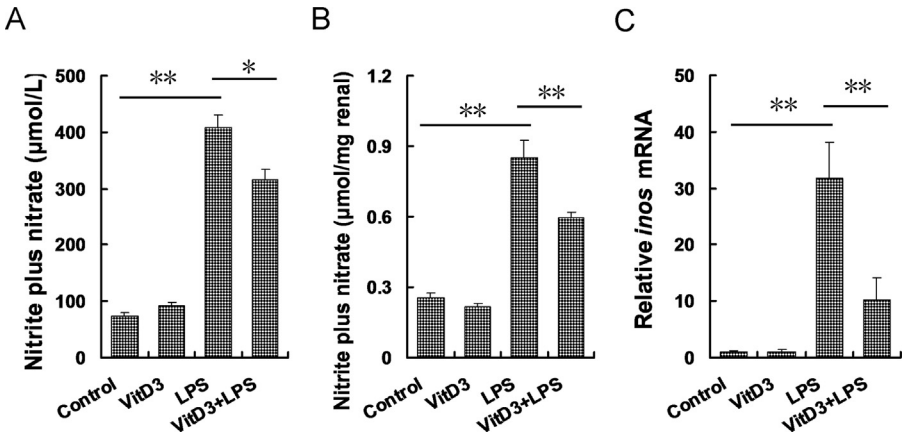

Fig. 5. Effects of VitD3 pretreatment on LPS-induced *inos* expression and NO production in kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Sera and kidneys were collected 6 h after LPS injection. (A) Serum nitrite plus nitrate level. (B) Renal nitrite plus nitrate level. (C) Renal *inos* mRNA was measured using real-time RT-PCR. All data were expressed as means ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

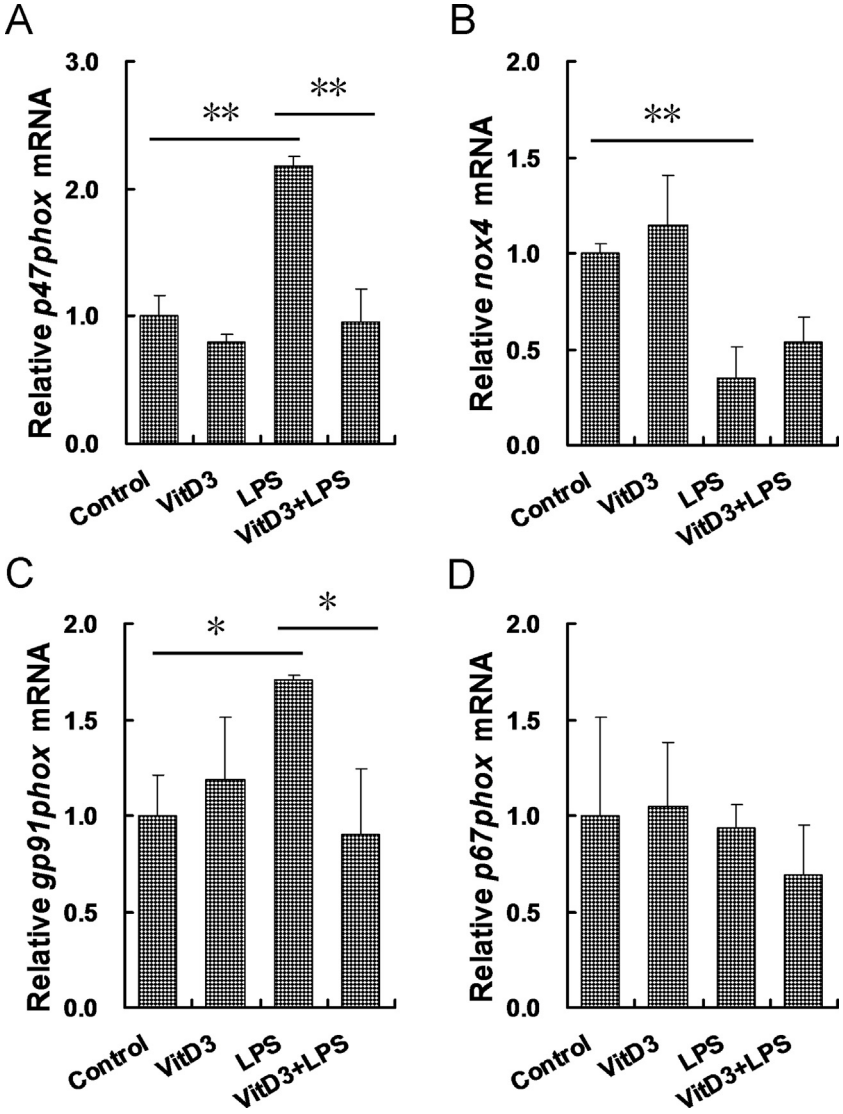

Fig. 6. Effects of VitD3 pretreatment on LPS-induced NADPH oxidase subunits in kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidneys were collected 6 h after LPS injection. Renal NADPH oxidase subunits were measured using real-time RT-PCR. (A) *p47phox*, (B) *nox4*, (C) *gp91phox*, (D) *p67phox*. All data were expressed as means ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

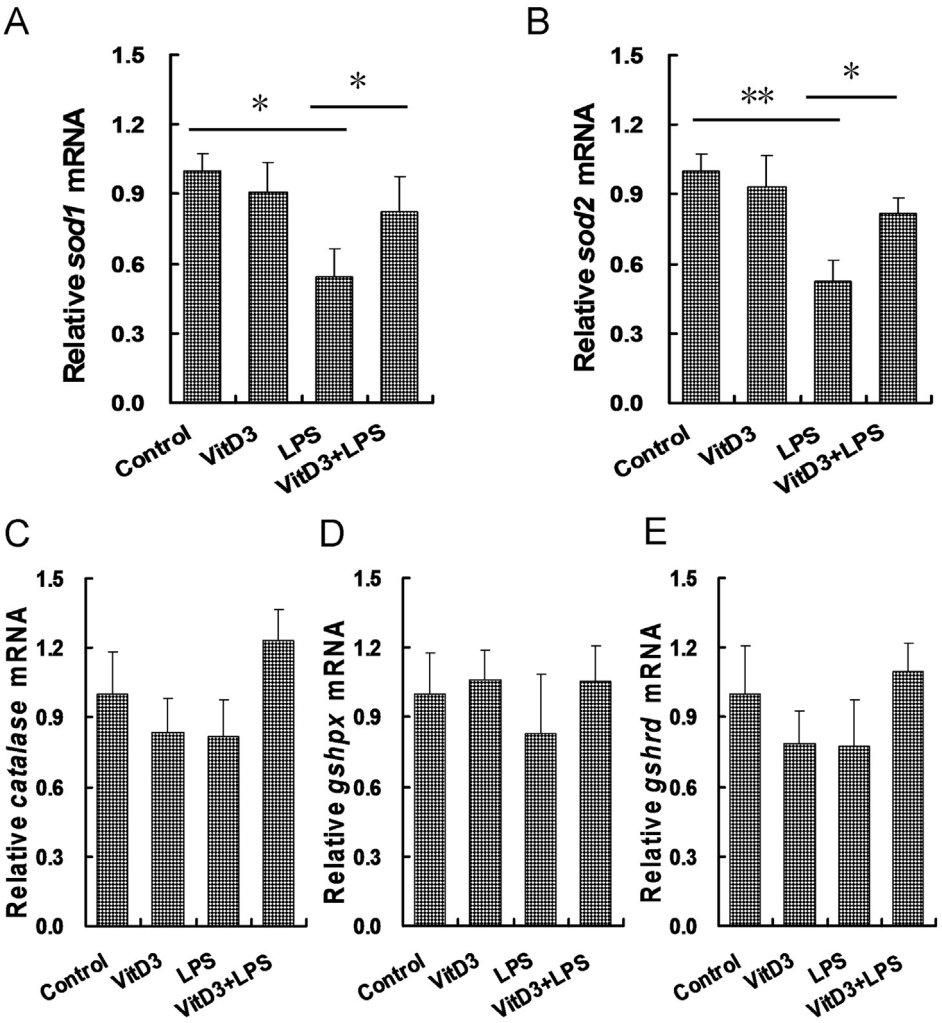

Fig. 7. Effects of VitD3 pretreatment on antioxidant enzyme genes in kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidneys were collected 1 h after LPS injection. Renal *sod1*,*sod2*, *catalase*, *gshpx* and *gshrd* mRNAs were measured using real-time RT-PCR. (A) *sod1*, (B) *sod2*, (C) *catalase*, (D) *gshpx*, (E) *gshrd*. All data were expressed as means ± SEM ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

4. Discussion

The present study found that VitD3 pretreatment prevented renal pathological damage in LPS-induced acute kidney injury. Moreover, VitD3 pretreatment significantly attenuated LPS-induced impairment for renal function. It is increasingly recognized that excess ROS production plays an important role in LPS-induced acute kidney injury [13,14]. Indeed, the present study found that renal GSH content was significantly reduced in a mouse model of LPS-induced acute kidney injury. In contrast, renal TBARS content, a marker of lipid peroxidation, was markedly elevated 6 h after LPS injection. In addition, strong 3-NT residues in tubular epithelial cells, a marker of protein nitration, was detected in LPS-treated mice. Interestingly, VitD3 pretreatment protected against LPS-induced renal GSH depletion and lipid peroxidation. In addition, VitD3 pretreatment significantly attenuated LPS-induced protein nitration in tubular epithelial cells. These results suggest that VitD3 pretreatment alleviates renal oxidative stress in LPS-induced acute kidney injury.

The mechanism through which vitamin D3 exerts its antioxidant activity remains obscure. Increasing evidence demonstrates that NADPH oxidases of the Nox family are important enzymatic sources of cellular ROS [31]. According to an earlier report, paricalcitol, a synthetic vitamin D analog, significantly ameliorated cardiac oxidative injury through reducing NADPH oxidase activity [32]. A recent study showed that calcitriol, the active form of vitamin D, markedly inhibited angiotensin II-induced ROS production and up-regulation of NOX-2 and NOX-4, two homologues in NADPH oxidases of the Nox family, in renal arteries [33]. The present study investigated the effects of pretreatment with VitD3 on the expression of renal NADPH oxidase subunits during LPS-induced acute kidney injury. As expected, renal *p47phox* and *gp91phox*, two NADPH oxidase subunits, were significantly up-regulated in LPS-treated mice. Interestingly, LPS-induced up-regulation of renal *p47phox* and *gp91phox* was normalized by VitD3 pretreatment. These results suggest that VitD3 alleviates LPS-induced renal oxidative stress through down-regulating oxidant enzyme genes.

A recent in vitro report showed that vitamin D significantly up-regulated the expression of glutamate cysteine ligase and elevated glutathione reductase activity in high-glucose exposed U937 monocytes [34]. According to another in vitro study, 1,25 (OH) 2D3 obviously up-regulated the expression of CuZn-SOD in endothelial cells [24]. The present study investigated the effects of VitD3 supplementation on renal antioxidant enzymes. As expected, renal *sod1* and *sod2*, two antioxidant enzyme genes, were significantly down-regulated 1 h after LPS injection. Although VitD3 alone had little effect on renal antioxidant enzymes,

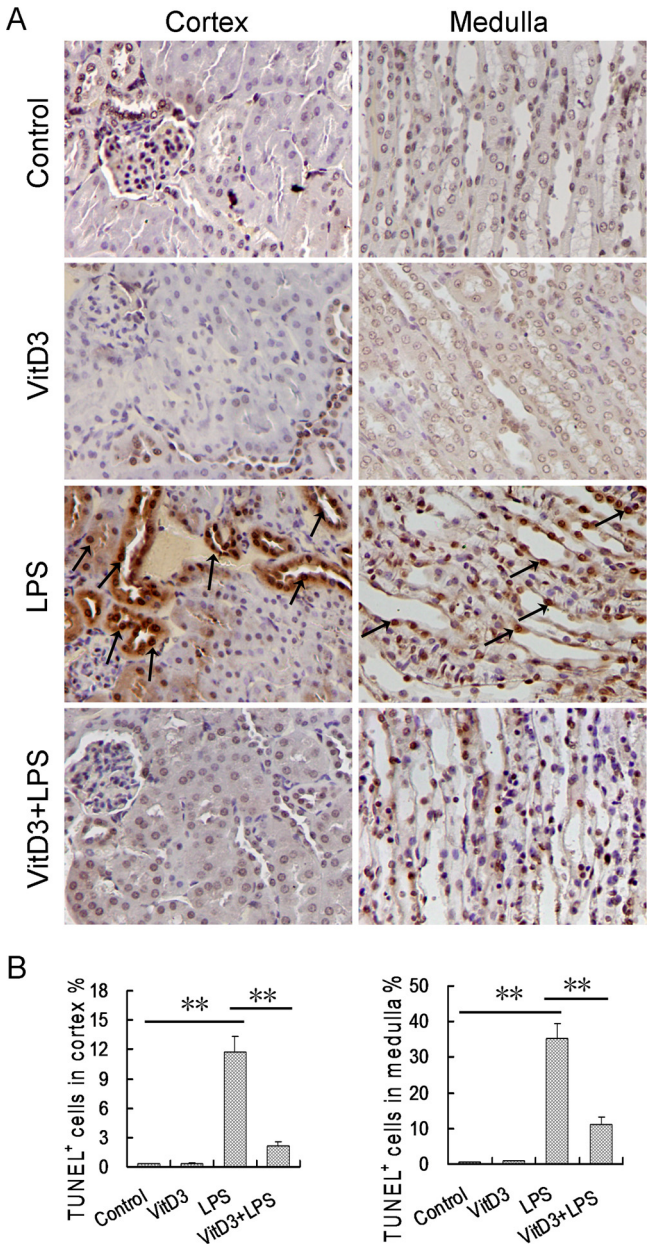

Fig. 8. Effects of VitD3 pretreatment on LPS-induced cell apoptosis in kidneys. In LPS group, mice were i.p. injected with LPS (2.0 mg/kg). In VitD3 + LPS group, mice were pretreated with three doses of VitD3 (25 μg/kg) at 48, 24 and 1 h before LPS injection. Kidneys were collected 6 h after LPS injection. (A) Cell apoptosis in kidney was detected by TUNEL. Arrows indicate TUNEL$^+$ cells in renal tubular cells. Representative photomicrographs from different groups were showed. Original magnification ×400. (B) TUNEL$^+$ cells were analyzed. All data were expressed as means ± SEM ($n = 6$). $^{**}P < 0.01$.

VitD3 pretreatment significantly attenuated LPS-induced down-regulation of *sod1* and *sod2* in kidneys. These results suggest that VitD3 alleviates LPS-induced renal oxidative stress through up-regulating antioxidant enzyme genes.

Increasing evidence demonstrates that iNOS-derived RNS is an important contributor to sepsis-induced acute kidney injury [35,36]. The present study investigated the effects of VitD3 pretreatment on renal *inos* expression in LPS-induced acute kidney injury. As expected, renal *inos* mRNA was up-regulated in LPS-induced acute kidney injury. Correspondingly, the levels of serum and renal nitrate plus nitrite were significantly elevated in LPS-treated mice. Although VitD3 alone had no effect on the expression of renal *inos*, LPS-induced renal *inos* up-regulation was markedly repressed in VitD3-pretreated mice. In addition, LPS-induced elevation of serum and renal nitrate plus nitrite was significantly attenuated by VitD3 pretreatment. These results suggest that VitD3 pretreatment inhibits renal *inos* up-regulation and renal NO production in LPS-induced acute kidney injury.

Several studies have observed a number of apoptotic tubular cells in LPS-induced acute kidney injury [37–39]. Indeed, VitD3 has an anti-apoptotic activity [40,41]. The present study investigated the effects of VitD3 pretreatment on LPS-induced tubular cell apoptosis in LPS-induced acute kidney injury. As expected, a number of apoptotic cells were observed in cortex and medulla when mice were injected with LPS. Interestingly, LPS-induced tubular cell apoptosis in cortex was almost completely blocked in VitD3-pretreated mice. In addition, LPS-induced tubular cell apoptosis in medulla was markedly attenuated by VitD3. These results suggest that VitD3 pretreatment inhibited LPS-induced tubular cell apoptosis in LPS-induced acute kidney injury. Thus, additional study is necessary to explore the mechanism of VitD3-mediated anti-apoptosis during LPS-induced acute kidney injury.

The protection of VitD3 pretreatment against LPS-induced acute kidney injury may have therapeutic implications. Several studies found that serum vitamin D level was negatively correlated with acute kidney injury in critically ill patients [42,43]. A recent report showed that vitamin D deficiency aggravated chronic kidney disease progression after ischemic acute kidney injury [44]. In addition, VitD3 protected mice from ischemia/reperfusion-induced acute kidney injury through suppressing TLR4-mediated inflammation [45]. The present study showed that oral administration with three daily recommended doses of VitD3 markedly elevated serum 25(OH)D concentration and efficiently activated renal VDR signaling. Importantly, VitD3 pretreatment obviously attenuated oxidative stress in LPS-induced acute kidney injury. Therefore, VitD3 may be used as a potential protective agent for clinical therapy especially in high-risk situations in which the patients are infected with bacteria.

The present study laid emphasis on the effects of VitD3 on renal oxidative stress in the pathogenesis of LPS-induced acute kidney injury. The present study has several limitations. First, the present study only investigated the effects of VitD3 pretreatment on renal oxidative stress in LPS-induced acute kidney injury. Second, the present study did not specify which ROS or RNS actually mediated the protective effects of VitD3 on renal oxidative stress in LPS-induced acute kidney injury. Third, the present study did not investigate the effects of VitD3 on renal oxidative stress in the pathogenesis of chronic kidney injury. Thus, additional work is required to determine which ROS or RNS actually mediated the protective effects of VitD3 pretreatment on renal oxidative stress in LPS-induced acute kidney injury. Moreover, it is required to explore the effects of VitD3 on renal oxidative stress in a model of repeated LPS administration. In addition, it is also necessary to explore the mechanism through which VitD3 regulates oxidant and anti-oxidant enzyme genes, especially the association between its antioxidant and anti-inflammatory activities.

In summary, the present study investigated the effects of VitD3 pretreatment on oxidative stress during LPS-induced acute kidney injury in mice. The present study indicates that VitD3 pretreatment alleviates LPS-induced renal GSH depletion and lipid peroxidation. In addition, VitD3 pretreatment attenuates LPS-induced serum and renal NO production and renal protein nitration. The present study demonstrates for the first time that VitD3 alleviates LPS-evoked renal oxidative stress through regulating oxidant and anti-oxidant enzyme genes in the kidneys. Thus, VitD3 pretreatment may have potential preventive and therapeutic utilities for protecting against sepsis-induced acute kidney injury.

Author contributions

Conceived and designed the experiments: DXX DXY. Performed the experiments: SX YHC ZXT CZ MZX HW. Analyzed the data: SX YHC HZ. Contributed reagents/materials/analysis tools: DXX DXY YHC. Contributed to the writing of the manuscript: DXX.

Competing interests

The authors have declared that no competing interests exist.

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