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TLR3 is involved in paraquat-induced acute renal injury

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ABSTRACT

Aims: To investigate the role of Toll-like receptor 3 (TLR3) in mouse paraquat-induced acute renal injury.
Materials and methods: Acute renal injury was established in C57BL/6J mice by intraperitoneal injection of paraquat (28 mg/kg). The mice were also injected intraperitoneally with TLR3 agonist poly I:C (20 mg/kg) or TLR3/dsRNA complex inhibitor (1 mg) 1 h before paraquat exposure. At 72 hour post paraquat exposure, the mice were sacrificed and the blood and renal tissues were collected to examine TLR3 expression in renal tissues, pathological injury in renal tissues, renal function, inflammation, and cell apoptosis.
Key findings: After paraquat exposure, TLR3 expression in mouse renal tissues was significantly increased, and pathological changes to the renal tissues and remarkable renal impairment were present. Compared to the paraquat group, the poly I:C group showed no significant difference in renal pathology, renal function, inflammation, or cell apoptosis. However, TLR3 inhibitor treatment significantly alleviated injury to the renal tissues, improved renal function, inhibited NF-κB activation, suppressed the infiltration of neutrophils, and lessened the expression of IL-1β, TNF-α, and keratinocyte chemoattractant (KC) in renal tissues. TLR3 inhibitor treatment also suppressed the activation of caspase-8 and caspase-3 and reduced apoptosis in the renal tissues.
Significance: Paraquat exposure significantly upregulates TLR3 expression in renal tissues, and activation of the TLR3 signaling pathway is an important contributor to paraquat nephrotoxicity. TLR3 activation exacerbates inflammation and cell apoptosis in renal tissues by activating NF-κB and caspase-8, thus promoting paraquat-induced acute renal injury.

1. Introduction

Paraquat is a bipyridyl quaternary ammonium salt herbicide that is widely applied in agricultural fields in various countries due to its herbicidal effect and automatic degradation in soil [1]. However, due to the lack of effective therapies, oral intake of 5–15 ml paraquat may be fatal, and the death rate after intake of 40–50 ml of paraquat may exceed 90% [2]. After entering the human body, paraquat is mainly excreted from the kidneys in its original form after filtration through the renal glomeruli and active secretion from the renal tubules. Thus, the kidneys are one of the organs which can contain the highest concentration of paraquat, which explains why remarkable acute renal injury occurs in the early stage of paraquat intoxication [3,4]. Acute renal injury greatly impacts paraquat excretion and can increase the paraquat concentration in the plasma by 5 times or more. This significantly increases paraquat toxicity and results in multiple organ dysfunction, therefore increasing patient mortality [5,6]. Therefore, it is essential to alleviate paraquat-induced acute renal injury and maintain good renal function in order to successfully treat paraquat poisoning.

Although many studies have examined paraquat's mechanism of action, the molecular mechanisms underlying paraquat toxicities have not yet been clarified. Toll-like receptors (TLRs) are an important family of receptors which recognize pathogen-associated molecular patterns (PAMPs) from microorganisms invading the human body and induce innate immune responses against the pathogens [7]. In mammals, TLRs also recognize host-derived molecules released from injured tissues and cells, which are called damage-associated molecular patterns (DAMPs) [8,9]. The binding of TLRs and their ligands plays a key role in the immune response induced by microorganism infection or tissue damage [10]. In previous studies, we have shown that the key adaptor myeloid differentiation factor 88 (MyD88), which is involved in the majority of TLR signaling pathways, is involved in paraquat-induced pulmonary inflammation and aggravates acute lung injury [11]. This suggests that several TLRs may participate in paraquat toxicity. However, TLR3 is a unique member of TLR family which transmits signals via recruitment of the adaptor TIR-domain-containing adapter-inducing interferon-β (TRIF) rather than MyD88 [12]. Therefore,

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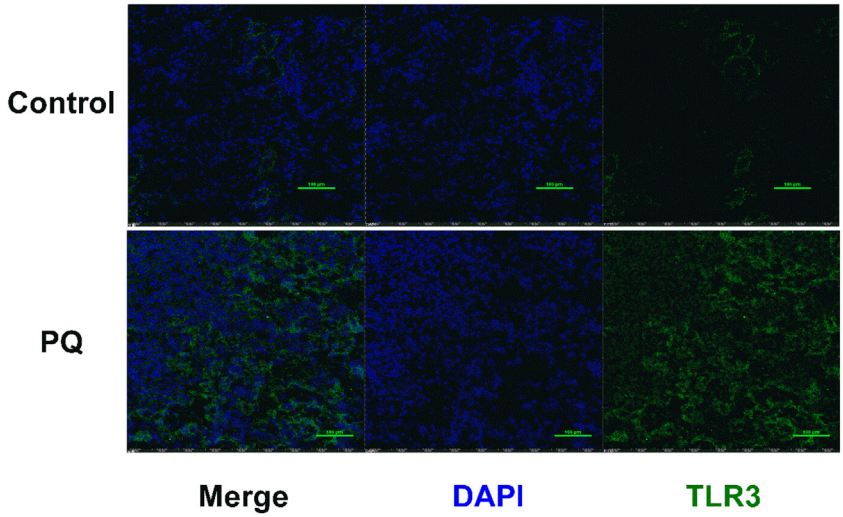

Fig. 1. TLR3 expression in renal tissues. In the control group (upper row), TLR3 was scarcely expressed in renal tissues. However, TLR3 was strongly expressed in the renal tissues of paraquat-exposed mice, and its expression was mainly concentrated in the tabular epithelial cells (lower row).

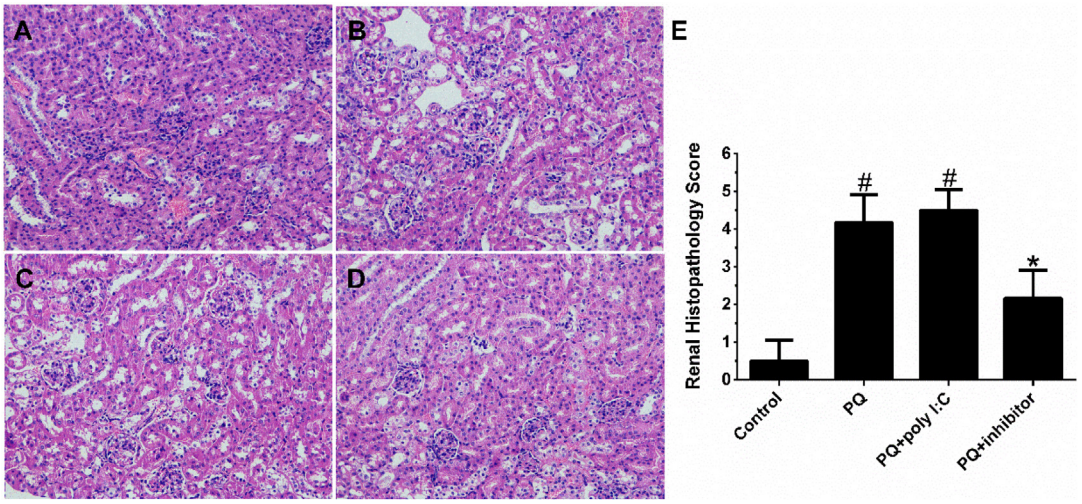

Fig. 2. Pathological injury of renal tissues is relieved by disrupting the TLR3 pathway. The kidney histological changes were determined by HE staining (A–D) and renal histopathological scoring (E) at 72 h post PQ exposure. A: control group, B: PQ group, C: PQ + poly I:C group, D: PQ + TLR3 inhibitor group. $^{\#}P < 0.05$ vs. control group, $^{*}P < 0.05$ vs. PQ group (n = 6).

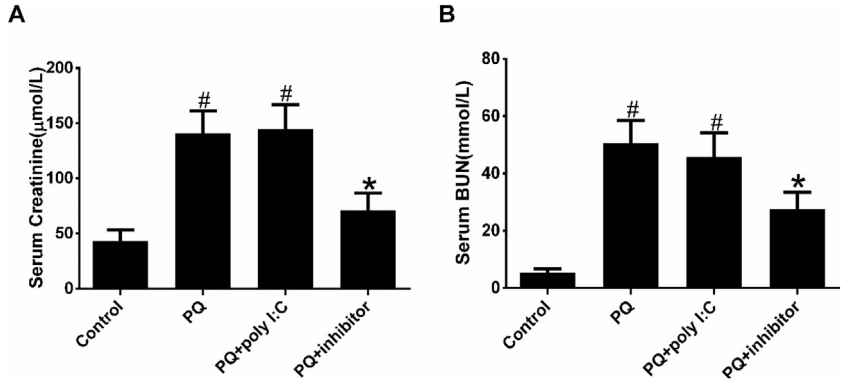

Fig. 3. Inhibiting the TLR3 pathway alleviated PQ-induced renal impairment. At 72 h after PQ exposure, blood samples were collected for analysis of renal function. TLR3 inhibitor markedly suppressed PQ-induced elevation of Serum BUN (A) and Creatinine (B) levels. $^{\#}P < 0.05$ vs. control group, $^{*}P < 0.05$ vs. PQ group (n = 6).

whether TLR3 is involved in paraquat toxicity is unknown. TLR3 is expressed in several tissues and organs, including pancreas, lungs, liver, heart, lymph nodes, and brain [13]. Recent studies have reported that TLR3 is similarly expressed in mouse and human renal tissues [14,15]. TLR3 mainly recognizes double-stranded RNA (dsRNA) released during virus replication [16], but a recent study showed that it can also

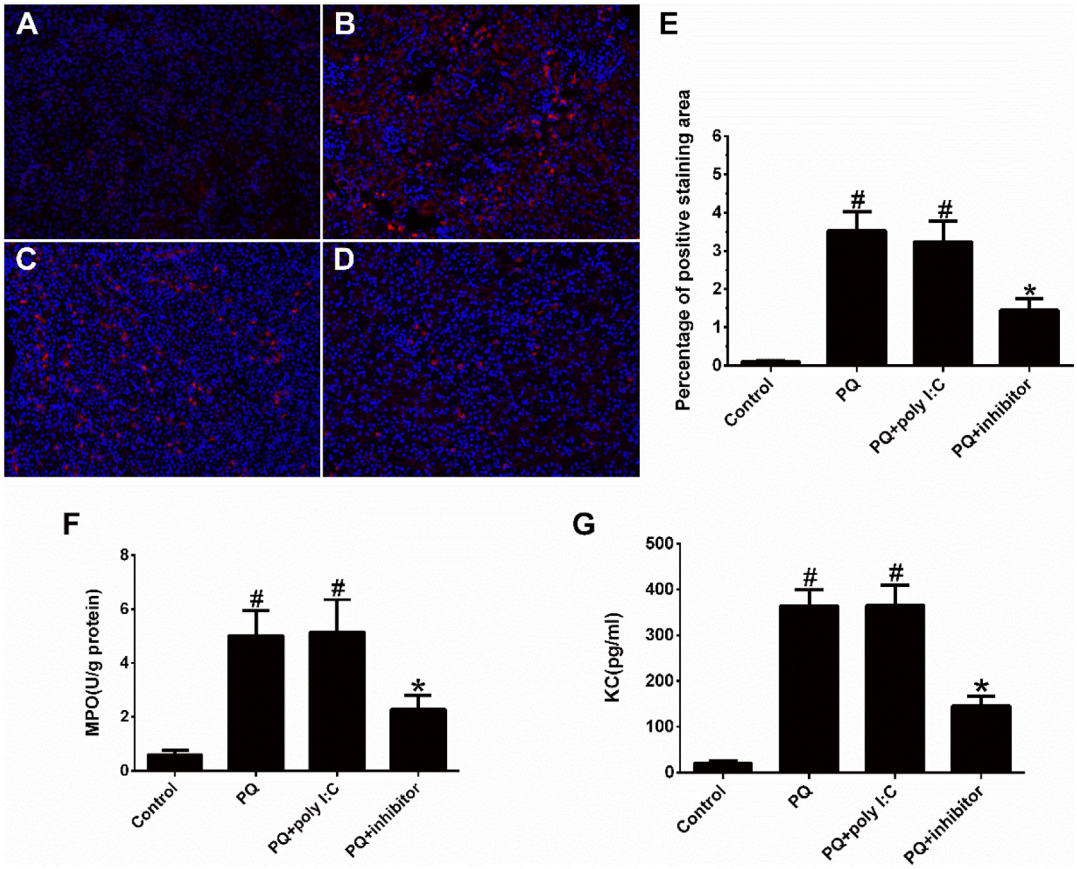

Fig. 4. Inhibiting the TLR3 pathway reduced neutrophils infiltration (A–E), MPO activity (F) and KC level (G) in renal tissues after PQ exposure. A: control group, B: PQ group, C: PQ + poly I:C group, D: PQ + TLR3 inhibitor group. $^{\#}P < 0.05$ vs. control group, $^{*}P < 0.05$ vs. PQ group (n = 6).

recognize mRNA or mRNA-protein complexes released by necrotic cells [9]. Numerous studies [17,18] have confirmed that paraquat induces apoptosis and necroptosis in several types of tissues and cells by catalyzing the production of reactive oxygen species (ROS). Therefore, the possibility of TLR3 activation during paraquat exposure cannot be excluded. Wörnle M et al. [19] and Paulus et al. [20] separately reported that TLR3 expression in renal tissues was significantly upregulated and that it promoted the development of renal injury in human hepatitis C-related glomerulonephritis and mouse ischemic reperfusion renal injury. Therefore, we hypothesized that TLR3 may participate in paraquat-induced acute renal injury. In order to validate this hypothesis, we detected TLR3 expression in the renal tissues of paraquat-exposed mice by immunofluorescence staining, and the results showed that TLR3 expression was significantly upregulated in paraquat-exposed mice. In order to investigate the role of TLR3 in paraquat-induced acute renal injury, we treated paraquat-exposed mice with the TLR3 agonist polyinosinic-polycytidylic acid (poly I:C) or TLR3/dsRNA complex inhibitor (*R*)-2-(3-chloro-6-fluorobenzo[*b*]thiophene-2-carboxamido)-3-phenylpropanoic acid. The results indicated that TLR3/dsRNA complex inhibitor treatment alleviated paraquat-induced acute renal injury. Our results show that TLR3 is involved in the process of paraquat-induced acute renal injury in mice and suggest that paraquat promotes the development of acute renal injury by activating the TLR3 signaling pathway.

2. Materials and methods

2.1. Animals

Wild-type male C57BL/6J mice of SPF grade (8–10 weeks, 20–25 g) were used in this study. All mice were purchased from the Animal Center of China Medical University. All animals were bred in the standard laboratory environment (12/12 hour light/dark cycle, temperature: 20–25 °C, relative humidity: 40–60%) and were allowed to have rodent animal diet and water at will. These experiments were approved by the Animal Ethics Committee of China Medical University.

2.2. Experimental protocol

The mice were randomly divided into 4 groups: 1) control group (n = 6): the mice were injected intraperitoneally with 0.1 ml PBS, and then with 0.1 ml physiological saline after 1 h; 2) paraquat group (n = 6): the mice were injected intraperitoneally with 0.1 ml PBS, and then with paraquat (28 mg/kg) after 1 h; 3) poly I:C group (n = 6): the mice were injected intraperitoneally with poly I:C (20 mg/kg), and then with paraquat (28 mg/kg) after 1 h; 4) TLR3 inhibitor (n = 6): the mice were injected intraperitoneally with 1 mg TLR3 inhibitor, and then with paraquat (28 mg/kg) after 1 h. At 72 hour post paraquat exposure, all the mice were sacrificed and the blood and renal tissues were collected for examination.

Paraquat and poly I:C were diluted into 0.1 ml with physiological saline and PBS, respectively, before use; and the TLR3 inhibitor was dissolved to 100 mM in DMSO and then diluted into 0.1 ml with PBS. In our previous study [21], we established a mouse acute renal injury model using 28 mg/kg paraquat. The degree of renal impairment in the mice was time-dependent, and the most significant renal impairment without death of the mice occurred at 72 hour post exposure. Therefore, we continuously used the 28 mg/kg dose in these experiments and sacrificed the mice 72 h after exposure. Poly I:C and TLR3 inhibitor were dosed as described previously [22,23]. Paraquat was bought from Sigma-Aldrich (St. Louis, MO, USA), poly I:C from Invivogen (San Diego, CA, USA), and TLR3 inhibitor from Calbiochem/EMD Millipore

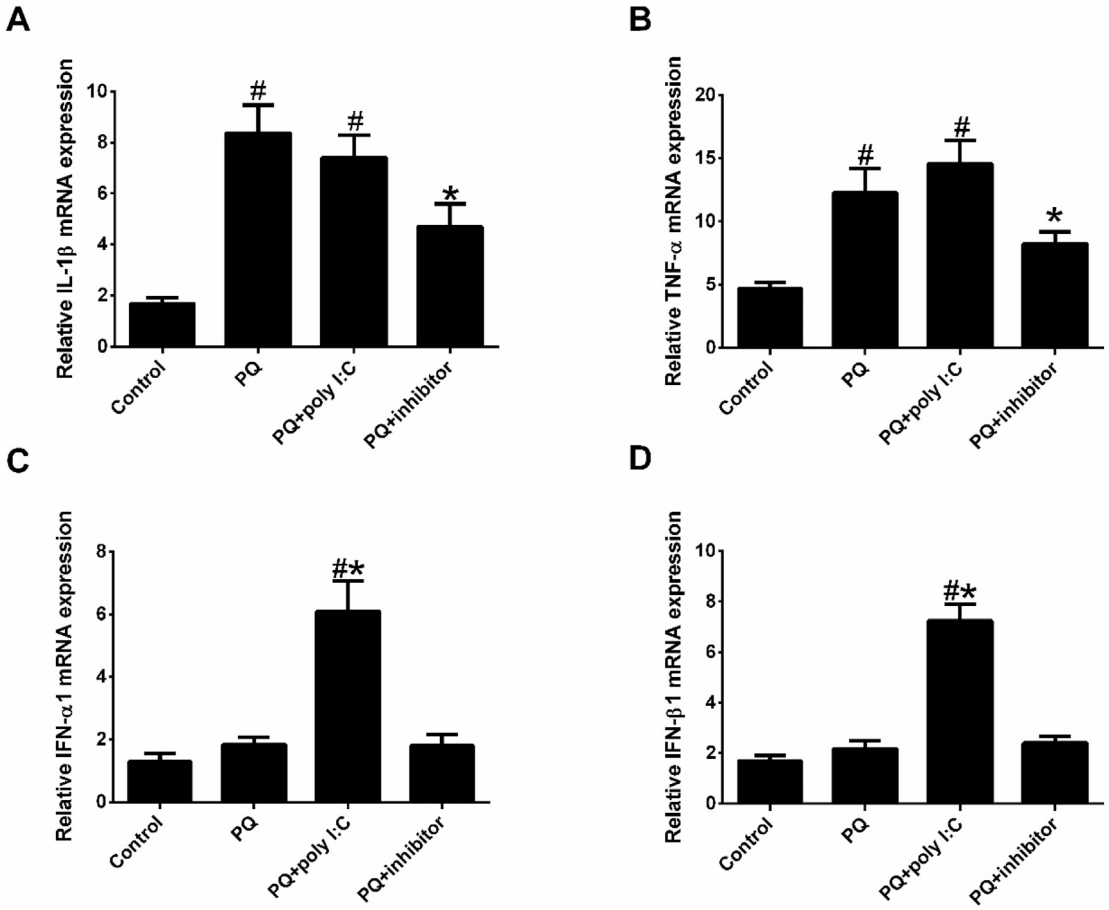

Fig. 5. Inhibiting the TLR3 pathway reduced cytokine expression in renal tissues. At 72 h after PQ exposure, renal tissues were collected to measure the mRNA levels of IL-1β (A), TNF-α (B), IFN-α1 (C) and IFN-β1 (D). $^{\#}P < 0.05$ vs. control group, $^{*}P < 0.05$ vs. PQ group (n = 6).

(Burlington, MA, USA).

2.3. Pathology of renal tissues

The renal tissues were fixed in 4% paraformaldehyde for 72 h, dehydrated in an alcohol gradient, embedded with paraffin, and then cut into 4-μm sections. The sections were deparaffinized in dimethylbenzene, stained with hematoxylin and eosin, and examined for histopathological changes using a light microscope. The pathological examination of sections by light microscopy was evaluated by the presence and severity of tubular degeneration, necrosis, protein casts, and dilated lumens. Histopathology scoring was as follows: 0, no injury; 1, minimal injury with < 10% cells; 2, mild injury with 10–25% cells; 3, moderate injury with 25–40% cells; 4, marked injury with 40–50% cells; 5, severe injury with 50% cells.

2.4. Detection of renal function

The blood samples were preserved overnight at 4 °C and centrifuged at 10,000 rpm for 10 min. The serum sample was then collected, and the creatinine and urea nitrogen levels were determined using a Colorimetric Assay Kit (Sigma-Aldrich, St. Louis, MO, USA). All experimental steps were performed according to the kit instructions.

2.5. Detection of MPO activity and keratinocyte chemoattractant (KC) content in renal tissues

Collect 50 μg renal tissues, cut into pieces in normal saline, and make a 10% tissue homogenate using a homogenizer. After centrifugation at 3000 rpm for 10 min, the supernatant was collected. We used a Colorimetric Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) to test MPO activity. In briefly, H_2O_2 and chromogenic reagent were added and the sample was incubated in water bath. The absorbance of the sample was measured at 460 nm (A460). One unit of MPO activity was defined as the amount of enzyme needed to degrade 1 μmol of H_2O_2 per min at 37 °C.

KC content was detected by an ELISA Kit (R&D Systems, Minneapolis, MN, USA). All steps were performed according to the corresponding kit instructions.

2.6. qRT-PCR

After the homogenization of renal tissues, RNA was extracted using Trizol reagent and then reverse transcribed into cDNA. cDNA was used as a template for PCR, and the following primers were used:

IFN-α1 F: 5′-GGACTTTGGATTCCCGCAGGAGAAG-3′,
IFN-α1 R: 5′-GCTGCATCAGACAGCCTTGCAGGTC-3′,
IFN-β1 F: 5′-CAGCTCCAAGAAAGGACGAAC-3′,
IFN-β1 R: 5′-GGCAGTGTAACTCTTCTGCAT-3′,
TNF-αF: 5′-GCAAGCTTCGCTCTTCTGTCTACTGAACTTCGG-3′,
TNF-αR: 5′-GCTCTAGAATGAGATAGCAAATCGGCTGACGG-3′,
IL-1β F: 5′-CGCAGCAGCACATCAACAAGAGC-3′,
IL-1β R: 5′-TCATCCTGGAAGGTCCACG-3′,

A Model 7500 Thermal Cycler (Applied Biosystems, Foster, CA, USA) was used for qRT-PCR, and all data were normalized by β-actin expression.

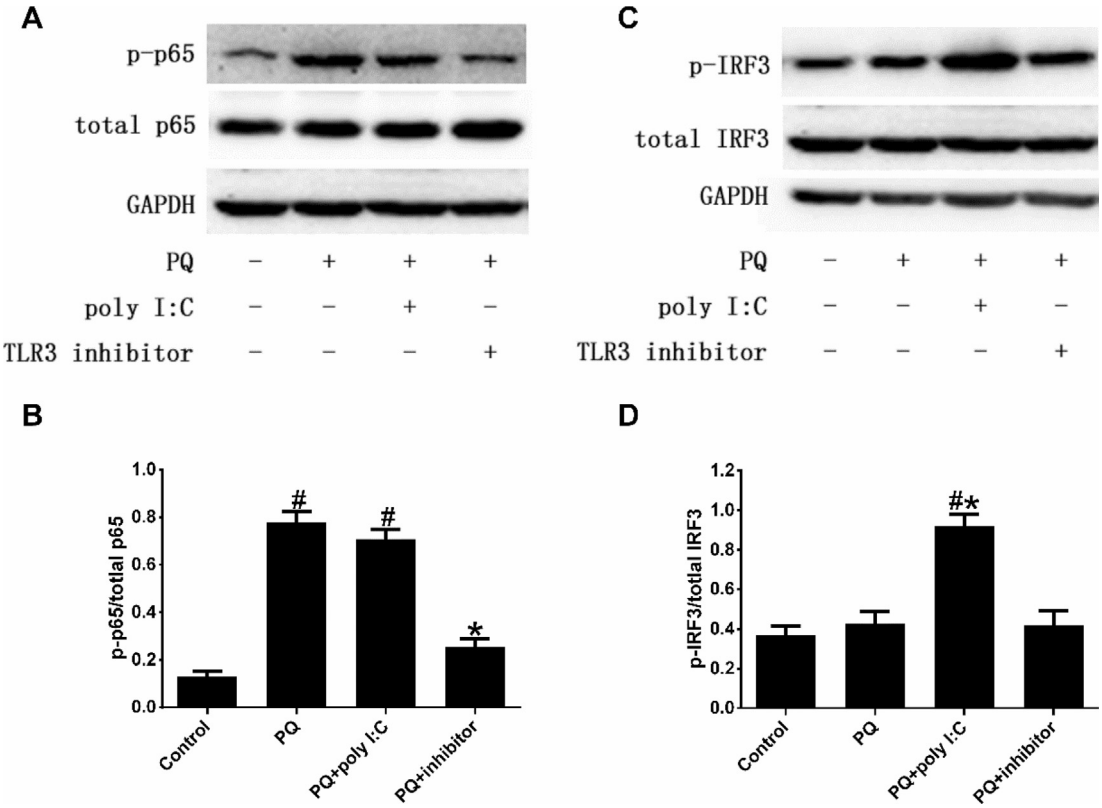

Fig. 6. The expression of NF-κB (A and B) and IRF3 (C and D) in renal tissues. #P < 0.05 vs. control group, *P < 0.05 vs. PQ group (n = 6).

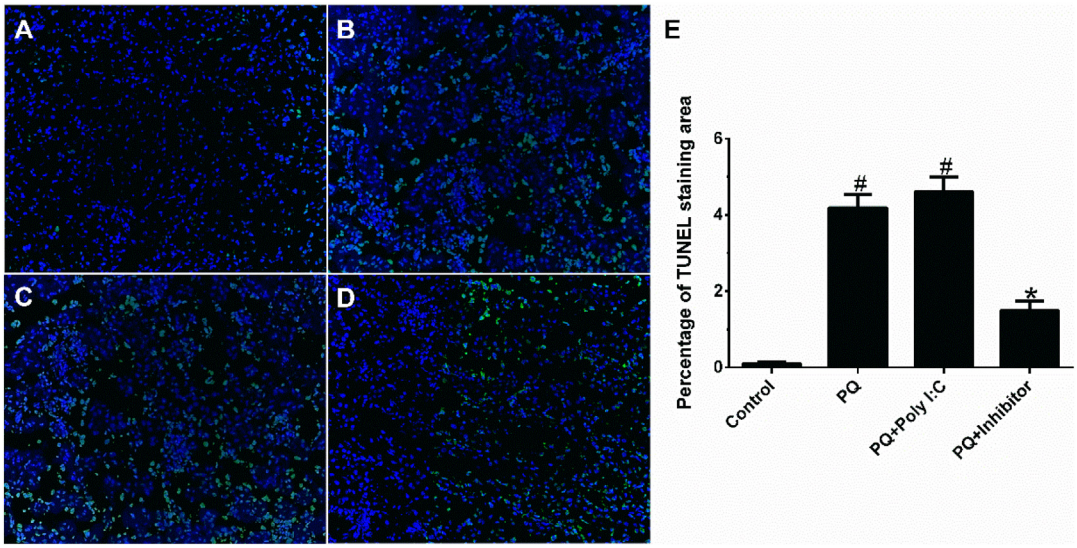

Fig. 7. Inhibiting the TLR3 pathway alleviated PQ-induced cell apoptosis in renal tissues. The apoptosis in renal tissues was determined by TUNEL staining (A–D) and percentage of TUNEL staining area (E). A: control group, B: PQ group, C: PQ + poly I:C group, D: PQ + TLR3 inhibitor group. #P < 0.05 vs. control group, *P < 0.05 vs. PQ group (n = 6).

2.7. Western blot

After the homogenization of renal tissues, total protein was extracted, the protein concentration was measured, and thereafter equal amounts of the protein samples were collected for 12% SDS-PAGE gel electrophoresis. After electrophoresis, the proteins were transferred onto a PVDF membrane. Then, the PVDF membrane was blocked with 5% skimmed milk for 1 h and incubated overnight at 4 °C with primary antibodies (total NF-κB p65 (1:1000), phospho-NF-κB p65 (1:2000), total IRF3 (1:1000), phospho-IRF3 (1:1000), caspase-8 (1:1000), caspase-3 (1:2000), RIP1 (1:1000), MLKL (1:1000), phospho-MLKL (1:1000)). After washing with TBST buffer, the PVDF membrane was incubated with secondary antibodies at room temperature for 1 h. After ECL luminescence, the results were analyzed using Gel-Pro-Analyzer 6.3. GAPDH or hsp70 was used as an internal reference. All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

2.8. Immunofluorescence

The renal tissues were embedded in OCT (optimal cutting

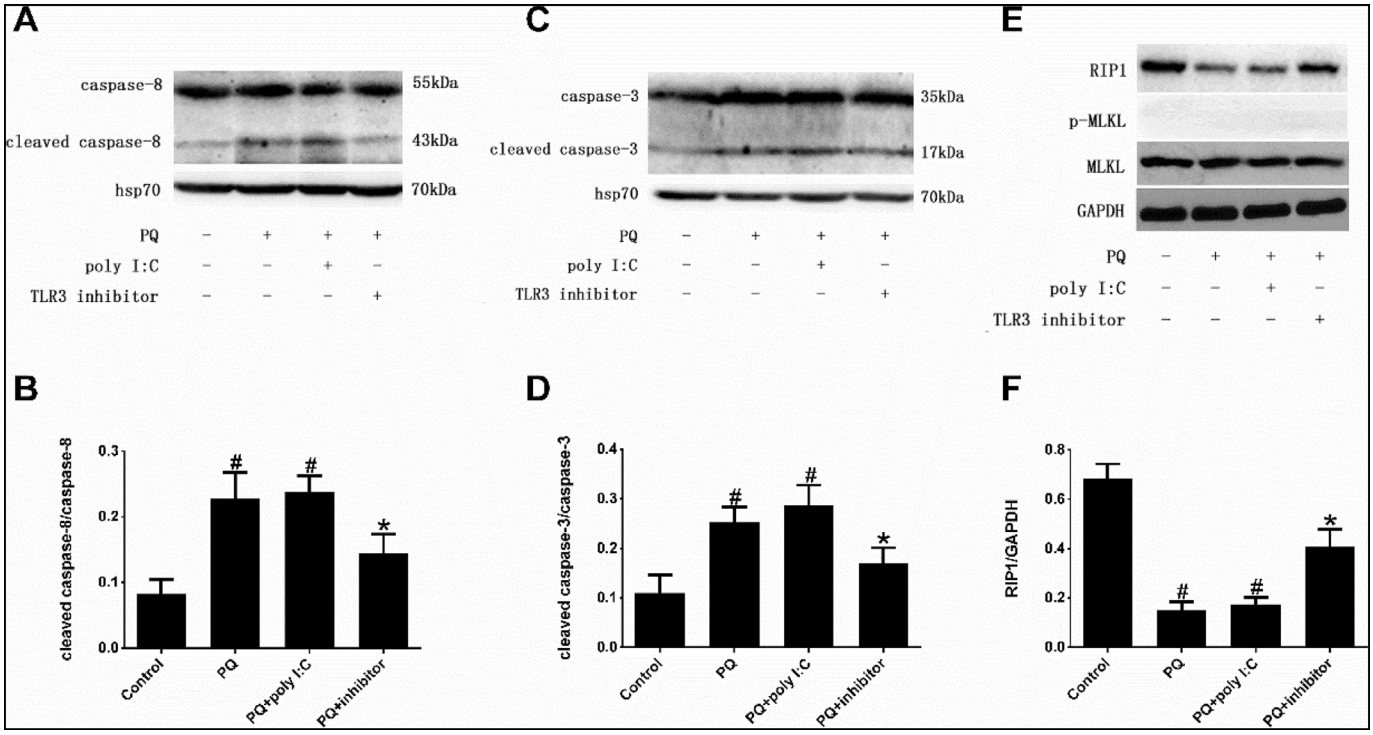

Fig. 8. The expression of caspase-8 (A and B), caspase-3 (C and D), RIP1 and MLKL (E and F) in renal tissues. $^{\#}P < 0.05$ vs. control group, $^{*}P < 0.05$ vs. PQ group (n = 6).

temperature) compound, frozen, and cut into 5 μm sections on a freezing microtome. After being frozen at −20 °C for 30 min, the sections were fixed in acetone for 10 min and then air-dried at room temperature. Then, for TLR3 staining, they were blocked with 10% fetal bovine serum (FBS) for 1 h, incubated with anti-TLR3 antibody (1:200, CST, Danvers, MA, USA) overnight at 4 °C, and subsequently incubated with FITC-labeled secondary antibody at room temperature for 1 h. For Ly-6G staining, the sections were blocked with 0.5% mouse BD Fc Block (San Jose, California, USA) for 30 min, incubated with Ly-6G antibody (1:200, eBioScience, San Diego, CA, USA) overnight at 4 °C, and subsequently incubated with Cy3-labeled secondary antibody for 1 h. Finally, these sections were mounted with the DAPI-containing anti-color fading reagent Prolong Gold (Invitrogen, San Diego, CA, USA) and observed under a fluorescence microscope.

2.9. TUNEL staining

The renal tissues were embedded with OCT (optimal cutting temperature) compound, frozen, and cut into 5 μm sections on the freezing microtome. The sections were stained using a TUNEL Kit (Promega, Fitchburg, WI, USA) according to the manufacturer's instructions, mounted with DAPI-containing Prolong Gold (Invitrogen, San Diego, CA, USA), and observed under a fluorescence microscope.

2.10. Statistical analysis

The data are presented as mean ± standard deviation (mean ± SD), and multiple comparisons were performed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

3. Results

3.1. TLR3 expression in the renal tissues of mice

In order to clarify whether the TLR3 signaling pathway is involved in paraquat-induced acute renal injury, we quantified TLR3 expression in renal tissues by immunofluorescence staining. In the control group, TLR3 was scarcely expressed in renal tissues. However, TLR3 was strongly expressed in the renal tissues of paraquat-exposed mice, and its expression was mainly concentrated in the tabular epithelial cells (Fig. 1). This indicated that paraquat exposure can significantly upregulate TLR3 expression in renal tissues.

3.2. Pathological injury of renal tissues is relieved by disrupting the TLR3 pathway

HE staining of renal tissues showed that the mice in the control group had normal renal tissue structure, complete glomerular and tabular structures, and regularly arranged nuclei of the tabular epithelial cells (Fig. 2A). In the paraquat and poly I:C groups, significant edema, detachment and necrosis of the renal tabular epithelial cells, and markedly dilated lumens were seen (Fig. 2B and C). No significant difference in the renal injury score was observed between the poly I:C and paraquat groups. TLR3 inhibitor treatment markedly alleviated the paraquat-induced renal injury; reduced edema, reduced detachment and necrosis of the renal tabular epithelial cells, and epithelium with a complete brush border were observed (Fig. 2D).

3.3. Paraquat-induced acute renal injury is relieved by inhibiting the TLR3 pathway

We evaluated the renal function of the mice by analyzing the serum creatinine and urea nitrogen levels. Compared to the control group, the serum creatinine and urea nitrogen levels in the paraquat group and the poly I:C group were significantly increased, and the use of TLR3 inhibitor markedly suppressed the elevation of these levels (Fig. 3).

3.4. The infiltration of neutrophils into the renal tissues was reduced by inhibiting the TLR3 pathway

We evaluated the infiltration of neutrophils into the renal tissues by immunofluorescence staining of neutrophil-specific Ly-6G. In the

paraquat and poly I:C groups, the Ly-6G staining area was significantly greater than that of the control group, and TLR3 inhibitor treatment inhibited the neutrophil infiltration (Fig. 4A–E). These results were confirmed by detection of MPO activity. Compared to the paraquat group, the MPO activity in the renal tissues was significantly decreased in the TLR3 inhibitor group (Fig. 4F). Infiltration of neutrophils into the tissue is closely related to the expression of the chemokine KC, thus we measured the levels of KC in the renal tissues. The KC levels were remarkably higher in paraquat and poly I:C groups than in the control group and were significantly lower in TLR3 inhibitor group than in the paraquat group (Fig. 4G).

3.5. Paraquat-induced inflammatory cytokine expression was inhibited by disrupting the TLR3 pathway

In the paraquat group and poly I:C group, the mRNA levels of IL-1β and TNF-α in the renal tissues were significantly higher than those in the control group. The mRNA levels of IFN-α and IFN-β in the poly I:C group were markedly greater than those in control group, and there was no significant difference in the expression of IFN-α and IFN-β between the paraquat group and the control group. The mRNA expression levels of IL-1β and TNF-α in the TLR3 inhibitor group were markedly lower than those in the paraquat group (Fig. 5).

3.6. TLR3 aggravated acute renal injury by activating NF-κB

To clarify the role of TLR3 in paraquat-induced acute renal injury, we detected the expression of two major pro-inflammatory transcriptional factors, NF-κB and IRF3, which act downstream of TLR3. Compared to the control group, NF-κB phosphorylation in the renal tissues was significantly increased in the paraquat and poly I:C groups, while TLR3 inhibitor treatment suppressed NF-κB phosphorylation (Fig. 6A and B). IRF3 phosphorylation was markedly higher in the poly I:C group than in the control group, but no significant difference was seen between the paraquat and control groups (Fig. 6C and D). This suggests that TLR3 promotes paraquat-induced acute renal injury by activating NF-κB but not IRF3.

3.7. Paraquat-induced cell apoptosis was reduced by disrupting the TLR3 pathway

We evaluated cell apoptosis in mouse renal tissues by TUNEL fluorescence staining. In the control group, there was nearly no TUNEL-positive staining in the mouse renal tissues. In the paraquat and poly I:C groups, the TUNEL-positive staining area was significantly higher than that of the control group. The apoptosis in the TLR3 inhibitor group was markedly lower than that of the paraquat group (Fig. 7).

To clarify the mechanism of paraquat-induced cell apoptosis, we measured caspase-8 and caspase-3 by Western blot. In the paraquat group and the poly I:C group, the levels of cleaved caspase-8 and cleaved caspase-3 in the mouse renal tissues were significantly greater than those in the control group, and they were markedly lower in the TLR3 inhibitor group than in the paraquat group. These results indicate that TLR3 induced cell apoptosis by activating the caspase-8 pathway (Fig. 8A–D).

To determine whether necroptosis is involved in the mechanism by which TLR3 aggravates renal injury, we examined the expression of RIP1 and MLKL by Western blot. After exposure to paraquat, the levels of RIP1 in paraquat group and the poly I:C group were significantly lower than those in the control group. Blocking TLR3 reduces the decline in RIP1 expression. In addition, no MLKL phosphorylation was observed in each group. These results suggest that TLR3 activation did not initiate cell necroptosis after PQ exposure (Fig. 8E and F).

4. Discussion

In this study, we found significant upregulation of TLR3 expression in mouse renal tissues after paraquat exposure. Furthermore, there was no remarkable difference in renal tissue injury and renal function between the paraquat group and poly I:C group, but TLR3 inhibitor treatment suppressed inflammation and cell apoptosis in the renal tissues and markedly relieved the pathological injury and changes in renal function induced by paraquat. These results suggest that the TLR3 signaling pathway is involved in the pathogenesis of paraquat-induced acute renal injury and promotes the development of acute renal injury by inducing inflammation and apoptosis.

TLR3, an important member of innate immune system, is expressed in several types of human tissues and cells. However, its expression level varies and can change rapidly in response to various stimuli including pathogens, inflammatory cytokines, and various stressors [24]. In this study, we observed similar conditions: TLR3 was scarcely expressed in mouse renal tissues from the control group but after paraquat exposure, TLR3 immunofluorescence staining in renal tissues greatly increased. TLR3 staining was mainly concentrated at the renal tabular epithelial cells, which is consistent with previous reports [20,25]. These findings have shown that paraquat can upregulate TLR3 expression in renal tissues, and this effect is independent of exogenous virus infection. TLR3 activation usually requires virus-derived dsRNA or artificially synthesized agonists like poly I:C [16], but previous studies have shown that endogenous nucleic acid products released after cell necrosis can similarly activate TLR3; and these interactions can be blocked by the TLR3/dsRNA complex inhibitor [22,23]. After entering the body, paraquat induces the production of numerous ROS, leading to cell necrosis [17,18]. Therefore, we hypothesize that TLR3 is also activated by endogenous nucleic acid products released after cell necrosis in our experimental model.

After activation, TLR3 recruits the downstream adaptor TRIF via its TIR structure to form a dimer, which induces a change in conformation that allows the entry of downstream signaling molecules into their binding sites. Subsequently, TRIF recruits TNF receptor-associated factor 6 (TRAF6) to form a complex containing TGF-β-activated kinase-1 (TAK1), TAK-1 binding protein 2 (TAB2), and protein kinase R, and then mediates the degradation of IκB-α and the activation of NF-κB [12,13]. In this study, we found that paraquat-induced NF-κB activation in renal tissues was significantly lessened by the TLR3 inhibitor, which indicates that paraquat may induce NF-κB activation via the TLR3-TRIF pathway. Activated NF-κB enters into the nucleus, and then binds to the κB unit on DNA to regulate the transcription of target genes (including inflammatory cytokines, chemotactic factors, and adhesion molecules), causing the dissemination and diffusion of the inflammatory reaction [26]. As observed in this experiment, the levels of inflammatory cytokines, chemotactic factors, and leukocyte count in the mouse renal tissues were markedly lower in the TLR3 inhibitor treatment group than in the paraquat group, which suggests that NF-κB plays an important role in paraquat-induced inflammation. In addition to activating NF-κB, the dimer formed by TLR3 and TRIF can recruit TRAF3, TRAF-family-member-associated NF-κB activator binding kinase 1 (TBK1), and inducible IκB kinase (IKKi) and induce IRF3 phosphorylation. Phosphorylated IRF3 enters into the nucleus to promote the transcription of interferon type I [27,28]. However, there was no significant difference in IRF3 phosphorylation levels or mRNA expression levels of IFN-α and IFN-β between the paraquat and control groups; these were higher in the poly I:C group than in paraquat group, but no remarkable difference was observed in renal tissue injury, renal function, and the level of inflammatory cytokines between the two groups. These results suggest that IRF3 and interferon type I are not involved in the mechanism of paraquat nephrotoxicity and that paraquat aggravates renal injury mainly via the TLR3-TRIF-NF-kB pathway.

In addition to promoting inflammation, TLR3 mediates exogenous signals to cause cell apoptosis. During this process, TRIF, the

downstream adaptor of TLR3, recruits receptor-interacting protein 1 (RIP1) and promotes its binding with Fas-associated protein with death domain (FADD) via its death domain (DD) at the C-terminus. Subsequently, FADD recruits and activates caspase-8 through the homotypic interaction of death effector domains (DEDs) between two proteins, and eventually activates caspase-3 to induce cell apoptosis [29,30]. In this experiment, we found that TUNEL positive staining in the renal tissues was far higher in the paraquat group than in the control group, and this effect was suppressed by the TLR3 inhibitor. This finding shows that paraquat can induce cell apoptosis by activating the TLR3 pathway. On the other hand, the TLR3-TRIF pathway can mediate necroptosis of cells when there is a lack of or suppression of caspase function [31]. RIP1 and RIP3 oligomerize via a common RIP homotypic interaction motif (RHIM)-dependent process leading to the phosphorylation of RIP3 in a RIP1 kinase-dependent manner [32]. Enforced dimerization of RIP3 can also directly lead to its autophosphorylation and activation of MLKL to lead to necrosis [33]. However, Caspase-8 prevents programmed necrosis by cleaving RIP1, separating the kinase and RHIM domains [34]. In the present experiment, we observed that the RIP1 levels significantly decreased after PQ exposure and no phosphorylation of MLKL was observed in all groups. In addition, the levels of cleaved caspase-8 and cleaved caspase-3 in mouse renal tissues were significantly higher in the paraquat group than in the control group, and remarkably lower in the TLR3 inhibitor treatment group than in paraquat group. These results suggest that after activating TLR3, paraquat worsens renal injury mainly through caspase-8-mediated cell apoptosis rather than necroptosis.

In conclusion, this study shows that paraquat exposure can significantly up-regulate TLR3 expression in renal tissues and can aggravate inflammation and apoptosis in renal tissues by activating the NF-κB and caspase-8 signaling pathways, thus promoting the development of acute renal injury. Our previous study showed that MyD88 played a similarly important role in the mechanism of paraquat toxicities [11], but inhibiting MyD88 would block all TLR pathways except TLR3, which would unavoidably lead to a risk of serious infection [35,36]. On the contrary, disrupting the TLR3 pathway would not significantly increase the risk of virus infection [37]. Therefore, a blockade of TLR3 is safer clinically and may provide a new strategy and target for the clinical treatment of paraquat poisoning.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

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