Paclitaxel Ameliorates Lipopolysaccharide-Induced Kidney Injury by Binding Myeloid Differentiation Protein-2 to Block Toll-Like Receptor 4–Mediated Nuclear Factor-κB Activation and Cytokine Production

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ABSTRACT

Recent studies suggest that paclitaxel, an anticancer agent, may modulate the injury and inflammatory responses in normal tissues. However, the underlying mechanism is not fully understood. Here we have examined the effect of paclitaxel on lipopolysaccharide (LPS)–induced acute kidney injury (AKI) in mice and further studied the mechanism. At relatively low doses, paclitaxel protected against LPS-induced AKI and improved animal survival. The beneficial effects of paclitaxel were accompanied by the downregulation of tumor necrosis factor-α, interleukin-1, and interleukin-6 production. In cultured renal tubular HK-2 cells, paclitaxel decreased the DNA-binding activity of nuclear factor-κB (NF-κB) during LPS treatment, inhibited the degradation of the inhibitor of κB-α, and blocked the expression and activation of NF-κB p65. At the upstream level, paclitaxel reduced LPS-induced association of myeloid differentiation protein-2 (MD-2) with Toll-like receptor 4 (TLR4). In an in vitro assay, paclitaxel was shown to directly bind recombinant MD-2. The inhibitory effect of paclitaxel on NF-κB activation and cytokine expression disappeared in MD-2 knockdown cells, indicating that paclitaxel acts through MD-2. Collectively, these results suggest that paclitaxel may bind MD-2 to block MD-2/TLR4 association during LPS treatment, resulting in the suppression of NF-κB activation and inhibition of proinflammatory cytokine production.

Introduction

Gram-negative bacterial infection associated with septic multiple organ failure remains a significant cause of morbidity and mortality in patients (Karima et al., 1999; Gao et al., 2008; Sharma et al., 2008). In kidneys, sepsis induces acute injury, tissue damage, and decrease of renal function, diminishing the efficacy of fluid supplementation and other therapeutics (Matejovic et al., 2011; Zarjou and Agarwal, 2011; Mayeux and MacMillan-Crow, 2012). Development of new drugs to treat septic acute kidney injury (AKI) therefore has a great potential to reduce mortality and morbidity in patients (Bagshaw et al., 2009; Zarjou and Agarwal, 2011). However, up-to-date, effective therapies for septic AKI are not available.

Lipopolysaccharide (LPS) is a major pathogenic factor during infection of Gram-negative bacteria (Mayeux, 1997; Karima et al., 1999; Bosshart and Heinzelmann, 2007). In mice and other animal models, LPS induces AKI, which is accompanied by a robust inflammatory response including leukocyte infiltration (Wang et al., 2009; Zahedi et al., 2010; Eadon et al., 2012). Proximal tubular cells are thought to play a role in the interstitial leukocyte infiltration in kidneys because of their anatomic proximity and ability to produce proinflammatory mediators and chemotactic cytokines (Danoff, 1998; Wan et al., 2008). In these cells, nuclear factor-κB (NF-κB), the ubiquitous proinflammatory transcription factor, has a pivotal role in the regulation of cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), and many early response genes during LPS treatment (Viedt et al., 2002; Zager et al., 2007; Wang et al., 2011).

Paclitaxel is an anticancer agent that stabilizes microtubules to interfere with microtubule breakdown during cell division or mitosis, resulting in cell cycle arrest and cell death in cancer (Kavallaris, 2010; Rao et al., 2012). However, recent research suggests that paclitaxel may modulate the injury and inflammatory response in nonmalignant tissues. For example, we showed that low-dose paclitaxel can efficiently

ABBREVIATIONS: AKI, acute kidney injury; BUN, blood urea nitrogen; ELISA, enzyme-linked immunosorbent assay; IL-1, interleukin-1; IL-6, interleukin-6; LPS, lipopolysaccharide; MD-2, myeloid differentiation protein-2; NF-κB, nuclear factor-κB; OSOM, outer stripe of the outer medulla; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-α.

reduce tubulointerstitial fibrosis in kidneys by inhibiting Smad2/3 activation and downregulating miR-192 (Zhang et al., 2010; Sun et al., 2011). In models of acute lung injury, paclitaxel may attenuate inflammation and vascular leak (Chausovsky et al., 2000; Mirzapoiazova et al., 2007). However, the molecular mechanism by which paclitaxel modulates inflammation is not fully understood. LPS is the primary ligand of Toll-like receptor 4 (TLR4), activating it through binding to its accessory protein myeloid differentiation protein-2 (MD-2) (Shimazu et al., 1999; Akashi et al., 2000). Interestingly, in in vitro binding assays, paclitaxel was shown to bind human and murine MD-2 (Zimmer et al., 2008). In view of these findings, this study was initiated to assess whether low doses of paclitaxel can block TLR4-mediated NF-κB signaling by binding to MD-2 leading to the attenuation of LPS-induced AKI.

Materials and Methods

Care and Use of Laboratory Animals

Animal experiments were performed in accordance with the regulations set by the Institutional Committee for the Care and Use of Laboratory Animals of Second Xiangya Hospital, China. C57BL/6 mice were housed on a 12-hour light/dark cycle, and were allowed free access to food and water.

Animal Model

The mice (male, aged 8–10 weeks) were divided into four groups: vehicle (saline) ($n = 24$), paclitaxel ($n = 24$), LPS ($n = 24$), and LPS plus paclitaxel ($n = 24$). LPS (from *Escherichia coli* O111:B4, 10 mg/kg; Santa Cruz Biotechnology, Santa Cruz, CA) was injected through the tail vein. Paclitaxel (0.15 mg/kg; Sigma-Aldrich, Shanghai, China) was injected via the jugular vein. In the LPS plus paclitaxel group, LPS and paclitaxel were injected simultaneously. The control group was administered with saline. Renal tissues were harvested for various biochemical and morphologic studies at 16 hours after LPS and/or paclitaxel treatment except for survival study.

Cell Culture and Grouping

Experiment Protocol 1. HK-2 cells were cultured in Dulbecco's modified Eagle's medium F-12 supplemented with 10% fetal bovine serum in an atmosphere of 5% CO$_2$–95% air at 37°C. The cells were plated into dishes and grown 24 hours to reach 90–100% confluence before being divided into four groups for the following treatments: control, paclitaxel, LPS (10 μg/ml), or LPS plus paclitaxel. After 2 or 24 hours of treatment, the cells were processed for various analyses. All experiments were carried out in quadruplicate.

Experiment Protocol 2. HK-2 cells were plated at approximately 70% density for transfection with MD-2 small interfering RNA (siRNA) (CGCAAAGAAGUUAUUUGCCGAGGAU) or control siRNA (CGCAAGAAUUGGUUUAGCCGAAGAU) (Invitrogen, Carlsbad, CA) using Lipofectamine 2000 for 24 hours. The effect of siRNA on the expression of MD-2 was verified by immunoblot analysis. After siRNA transfection, the cells were cultured for 24 hours to reach confluence. The cells were then treated with LPS and/or paclitaxel for another 24 hours in the culture medium with 20 mM of sodium lactate.

Measurement of TNF-α, IL-1, IL-6, Serum Creatinine, and Blood Urea Nitrogen

Blood samples and kidney tissues were collected from mice during the in vivo experiment and culture medium was collected during the cell culture study. The levels of TNF-α, IL-1, and IL-6 were determined by using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Inc, Minneapolis, MN). In all cases, a standard curve was constructed from standards provided by the manufacturers. Renal cytokine levels were normalized to protein concentration in lysate. Blood urea nitrogen (BUN) and creatinine levels were measured using an AutoAnalyzer (Roche Diagnostics, Mannheim, Germany).

Antibody-Sandwich ELISA Analysis of Paclitaxel Binding to MD-2

Recombinant human MD-2 was produced in *E. coli* as described previously (Gruber et al., 2004). The method of antibody-sandwich ELISA for the detection of binding of paclitaxel to MD-2 was modified from a previous study (Resman et al., 2008). This method is based on the use of a mouse monoclonal antibody that binds to free MD-2, but not to LPS-bound MD-2. All antibodies in this assay were purchased from Majorbio BioTech Co. Ltd. (Shanghai, China). Briefly, the wells of a microtiter plate were precoated with a chicken polyclonal anti-MD-2 antibody. Recombinant MD-2 was added into the wells at 2 μM for 2 hours of incubation. Then 0–2 μM paclitaxel was added into the wells for another 2 hours of incubation. Finally, a mouse anti-MD-2 antibody and a goat anti-mouse IgG conjugated with horseradish peroxidase were added to the wells. After extensive washes, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS; a horseradish peroxidase substrate) was added to detect absorbance at 420 nm.

Immunoprecipitation

Approximately 500 μg cellular protein was immunoprecipitated with 2 μg antibodies to TLR4 (gift from Dr. Ruslan Medzhitov, Yale University, New Haven, CT) for 1 hour at 4°C followed by the addition of 15 μl protein A/G-agarose beads (Santa Cruz Biotechnology) as previously described (Ha et al., 2010). The precipitates were washed 4 times with lysis buffer and subjected to immunoblotting for MD-2 and TLR4.

Histology

For histologic analysis, kidney tissues fixed with 4% buffered paraformaldehyde were embedded in paraffin, and 3-μm-thick sections were prepared. The sections were then stained with hematoxylin and eosin. Histologic changes in the cortex and the outer stripe of the outer medulla (OSOM) were scored by a blinded observer. The percentage of tubules that displayed cellular necrosis, loss of brush border, cast formation, vacuolization, and tubule dilation were scored as follows: 0 = none, 1 = <10%, 2 = 11–25%, 3 = 26–45%, 4 = 46–75%, and 5 = >76% (Miyaji et al., 2003; Brooks et al., 2009).

Immunoblot Analysis

A standard protocol was followed for immunoblot analysis. Briefly, cells were treated with a lysis buffer (Sigma-Aldrich) containing phosphatase inhibitors (Calbiochem, Beijing, China). Samples (20 μg) were resolved by SDS-PAGE and then transferred onto nitrocellulose membrane (Amersham International, Buckinghamshire, UK) for sequential incubations with primary and secondary antibodies. Antibodies used included the following: mouse monoclonal anti-NF-κB p65, rabbit polyclonal anti-p-NF-κB p65, rabbit polyclonal anti-TLR4, peroxidase-conjugated goat anti-mouse, and goat anti-rabbit IgGs were purchased from Santa Cruz Biotechnology. Mouse monoclonal anti-MD-2 and anti-inhibitor of κB-α antibodies were from Abcam (Cambridge, MA).

Statistical Analysis

Data were calculated as mean ± S.E.M. One-way analysis of variance followed by Tukey's post hoc test was used to compare multiple treatment groups. Two-way analysis of variance was used to assess the statistical significance of the differences between multiple treatment groups at different time points. Kaplan–Meier analysis (log-rank test) was used to record animal survival. Statistical significance was set at $P < 0.05$.

Results

Paclitaxel Improves Animal Survival After LPS Treatment

LPS treatment induced animal death within 1 day; by the end of 4 days, only 20–30% of animals survived. Cotreatment with 0.15 mg/kg paclitaxel delayed animal death by 8 hours; notably, the majority (68%) of animals survived in this group at the end of 4 days (Fig. 1).

Paclitaxel Reduces Kidney Injury and Renal Dysfunction in LPS-Treated Mice

LPS-induced kidney injury was shown in the tubules in both renal cortex and OSOM. This tubular damage was significantly ameliorated by paclitaxel (Fig. 2, A and B). To note, the tubular damage observed in this study appeared more severe than that of previous studies (Wu et al., 2007; Takahashi et al., 2012). Although the cause of the severity is unclear, it may be related to the LPS dosage tested and the animal husbandry condition. To analyze kidney function, BUN and serum creatinine were determined. As shown in Fig. 2C, LPS induced significant increases in both BUN and serum creatinine levels. The increases were significantly attenuated by cotreatment with paclitaxel.

Paclitaxel Decreases Serum and Renal TNF-α, IL-1, and IL-6 Levels in LPS-Treated Mice

The effects of paclitaxel on serum and renal TNF-α, IL-1, and IL-6 were determined by ELISA. As shown in Fig. 3, LPS induced marked increases in the levels of TNF-α, IL-1, and IL-6, which were suppressed by paclitaxel. Paclitaxel had no effects on TNF-α, IL-1, and IL-6 expression under control conditions.

Paclitaxel Attenuates LPS-Induced NF-κB Activation in Renal Tubular Epithelial Cells

To understand the mechanism whereby paclitaxel blocks LPS-induced cytokine production (Fig. 3), we examined human renal tubular HK-2 epithelial cells after LPS/paclitaxel treatment. Within 24 hours, 2 μM paclitaxel did not have significant effects on the viability of HK-2 cells as shown by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
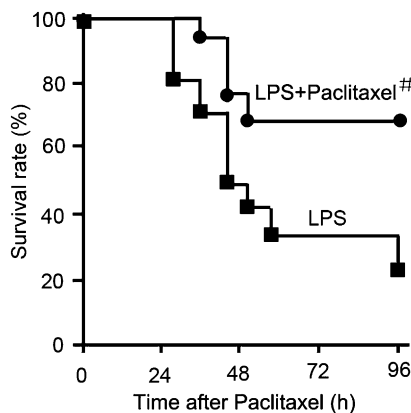
cell viability analysis (Fig. 4A). ELISA analysis showed that LPS induced NF-κB DNA-binding activity in HK-2 cells and notably, this effect was markedly reduced by paclitaxel (Fig. 4B). Furthermore, LPS induced the expression of phospho-p65, p65, and TLR4 expression, which was also reduced by paclitaxel (Fig. 4C). Consistently, expression of the inhibitor of κB was decreased during LPS treatment and this decrease was prevented by paclitaxel (Fig. 4C). These results were verified by densitometry analysis of the immunoblots (Fig. 4, D–G).

Paclitaxel Attenuates LPS-Induced Interaction between MD-2 and TLR4

A critical upstream event leading to NF-κB activation during LPS treatment is the activation and binding of MD-2 with TLR4. Therefore, we further examined the effect of paclitaxel on LPS-induced MD-2/TLR4 association by co-immunoprecipitation assay. Briefly, cell lysate was immunoprecipitated with an anti-TLR4 antibody, followed by immunoblot detection of MD-2 in the immunoprecipitates. As shown in Fig. 5A, LPS stimulation significantly increased the association between MD-2 and TLR4, which was reduced
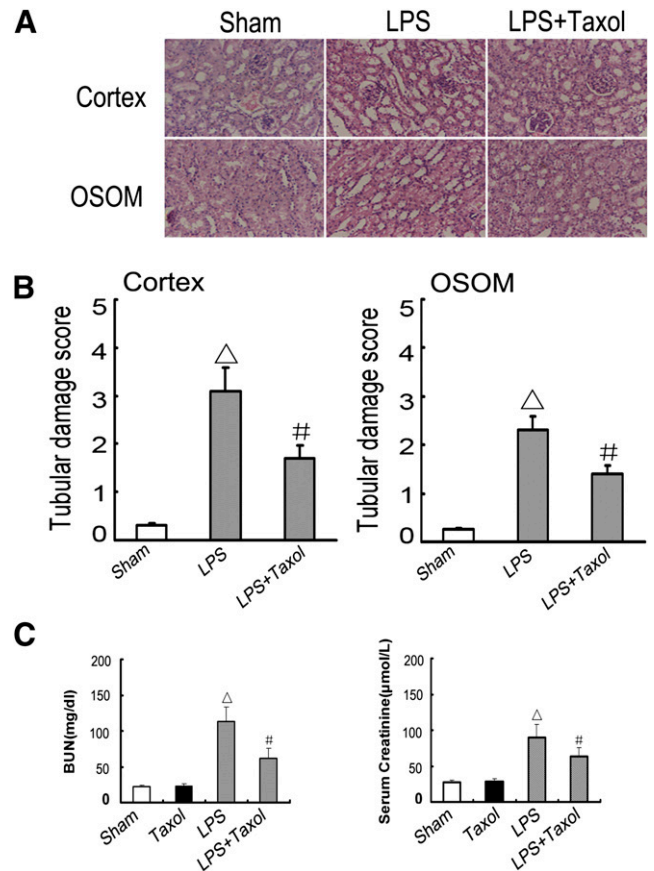
Fig. 2. Effect of paclitaxel on LPS-induced renal tissue damage, BUN, and serum creatinine in mice. Male C57 mice were injected with 10 mg/kg LPS with or without 0.15 mg/kg paclitaxel. As a control, the sham group was injected with saline. Kidney tissues and blood samples were collected 16 h after treatment. (A) Kidney sections were stained with hematoxylin and eosin to review tubular damage. (B) Tubular damage scores of kidney cortex and OSOM. (C) BUN and serum creatinine. Data are shown as mean ± S.E.M. (n = 12). $^{\triangle}P$ < 0.05 versus the sham group; $^{\#}P$ < 0.05 versus the LPS group. Original magnification, ×200 in A.
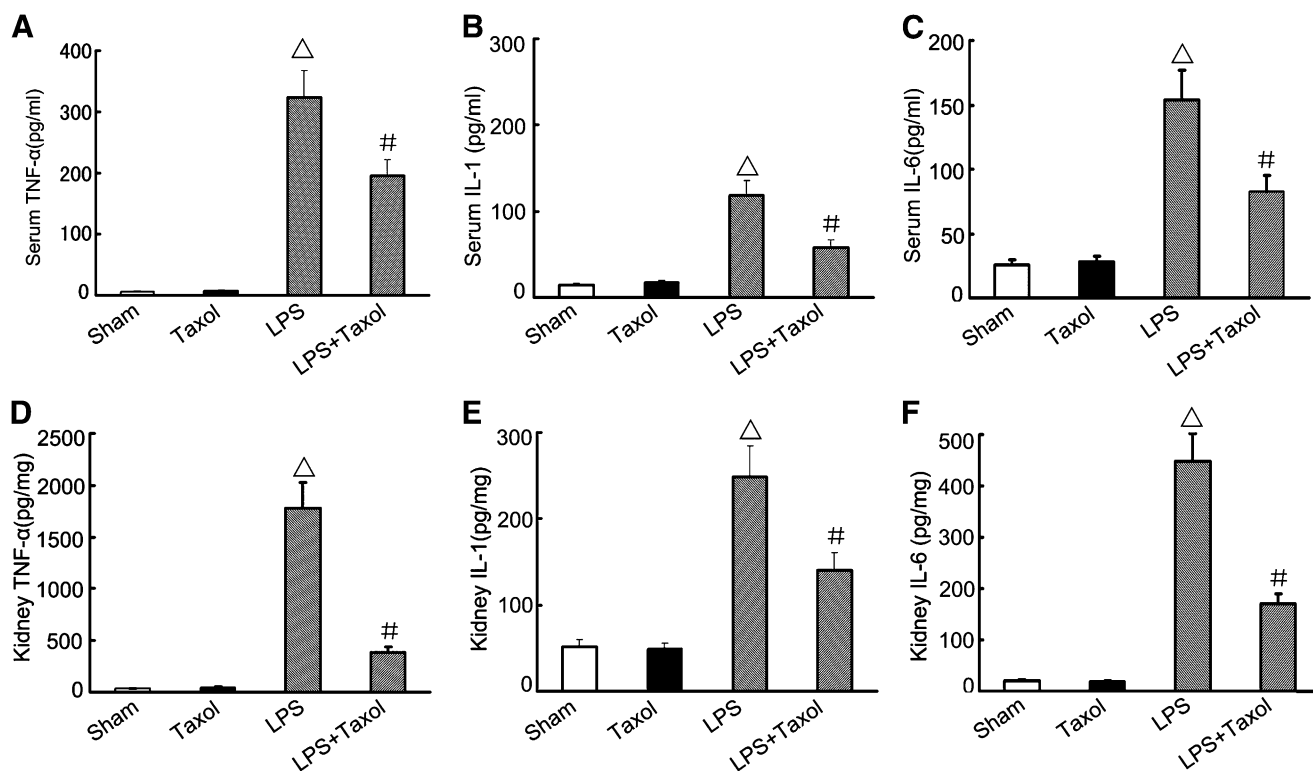
Fig. 1. Effect of paclitaxel on animal survival after LPS treatment. Male C57 mice were injected with 10 mg/kg LPS with or without 0.15 mg/kg paclitaxel. Animal survival was recorded at indicated time points. $^{\#}P$ < 0.05 versus the LPS group (n = 12).

Fig. 3. Effect of paclitaxel on LPS-induced expression of TNF-α, IL-1, and IL-6 in serum and kidney tissues. Male C57 mice were injected with 10 mg/kg LPS with or without 0.15 mg/kg paclitaxel, whereas the sham group was injected with saline. Kidney tissues and blood samples were collected 16 hours after treatment of ELISA determination of TNF-α, IL-1, and IL-6. Data are presented as mean $\pm$ S.E.M. ($n = 12$). $^{\triangle}P < 0.05$ versus the sham group; $^{\#}P < 0.05$ versus the LPS group.

by paclitaxel. As a control, the immunoprecipitation by a control IgG did not result in precipitation of MD-2 (not shown). One possibility is that paclitaxel may block MD-2/TLR4 association by directly binding MD-2. To test this, we determined the binding of paclitaxel to MD-2. For this purpose, recombinant human MD-2 was incubated with a monoclonal antibody that binds to free MD-2, but not to paclitaxel-bound MD-2. In this assay, paclitaxel was shown to reduce the association of MD-2 with its antibody (Fig. 5C), indicating that paclitaxel indeed binds to MD-2.

### The Anti-Inflammatory Effect of Paclitaxel Depends on MD-2

To determine whether the anti-inflammatory effect of paclitaxel depends on MD-2, we downregulated MD-2 with specific siRNA. As shown in Fig. 6A, transfection of HK-2 cells with MD-2 siRNA led to a marked MD-2 downregulation. Importantly, in MD-2 siRNA transfected cells, the stimulatory effects of LPS on NF-κB DNA-binding activity and cytokine expression were diminished. In addition, paclitaxel did not show further inhibitory effects in these cells (Fig. 5C), suggesting that the anti-inflammatory effect of paclitaxel depends on MD-2.

Discussion

Septic AKI is a major clinical problem that leads to significant mortality. Currently, there are no effective therapies. In this study, we have demonstrated that the cancer therapy drug, paclitaxel, has protective effects on LPS-induced AKI

and improves survival. LPS is the main endotoxin produced by Gram-negative bacteria, but it does not represent the entire spectrum of pathogenic factors in sepsis. In designing renoprotective therapeutics for the treatment of AKI, it is also important to consider both injury and subsequent kidney repair. Ideally, the therapeutics can limit AKI at the early phase and promote kidney repair and/or prevent fibrosis at the progression phase. In this regard, paclitaxel may adversely affect kidney repair, but this will certainly depend on the dosage used and the pharmacokinetics of paclitaxel. Further preclinical and clinical studies may need to examine the usefulness of paclitaxel in the treatment of septic AKI.
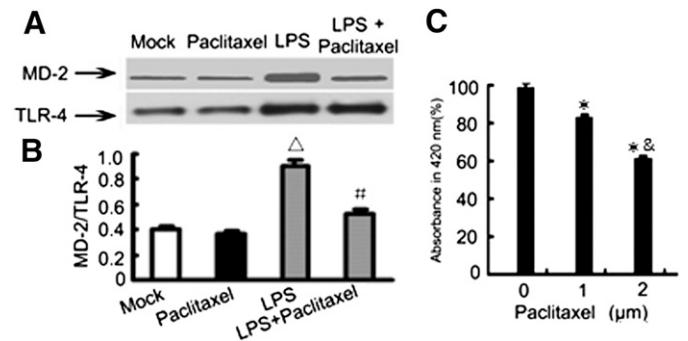
In LPS-treated mice, our ELISA assay demonstrated the inhibitory effect of paclitaxel on the production of proinflammatory cytokines, including TNF-α, IL-1, and IL-6. TNF-α and related cytokines are known to contribute to the toxic effect of LPS (Beutler and Kruys, 1995). However, it is controversial regarding the effects of paclitaxel on the expression of proinflammatory factors. In isolated murine macrophages, paclitaxel can increase TNF-α and IL-1 release (Ding et al., 1990; Bogdan and Ding, 1992). In contrast, taxanes (paclitaxel and docetaxel) decrease the levels of IL-1 and TNF-α and increase levels of interferon-γ, IL-2, and IL-6 in the serum of breast cancer patients (Tsavaris et al., 2002). In LPS-induced lung injury, Mirzapoiazova et al. (2007) demonstrated that paclitaxel may reduce the serum levels of cytokines including TNF-α and IL-6. Our data are consistent with the latter studies. Although the exact reason underlying the result discrepancy between these studies is unclear, it
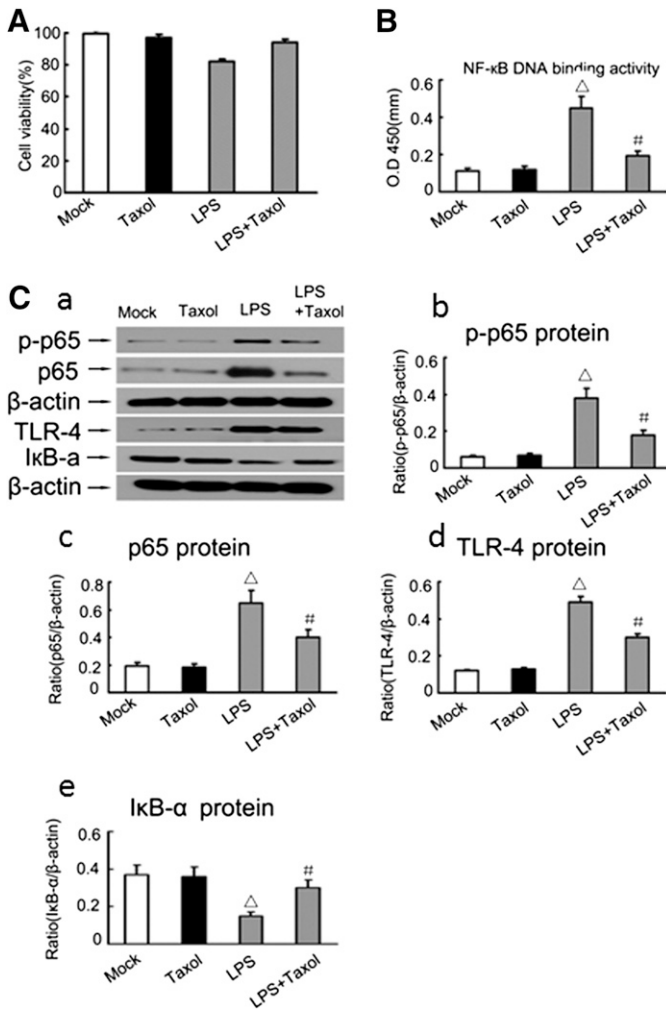
Fig. 4. Effect of paclitaxel on LPS-induced activation of p65, degradation of IκBa, and expression of TLR4 in HK-2 cells. HK-2 cells were treated with 10 μg/ml LPS in the presence or absence of 2 μM paclitaxel. (A) Cell viability determined by MTT assay at 24 hours of treatment. (B) NF-κB DNA-binding activity determined by ELISA assay at 2 hours of treatment. (C) Immunoblot analysis of phospho-p65, p65, IκBa, and TLR4 expression in HK-2 cells at 2 hours of treatment. (a) Representative immunoblot. (b,c,d,e) Results of densitometric analysis. Data are presented as mean ± S.E.M. ($n = 6$). $^\triangle P < 0.05$ versus the mock group or paclitaxel-only group; $^\# P < 0.05$ versus the LPS group. IκB-α, inhibitor of κB-α.

Fig. 5. (A) Paclitaxel blocks LPS-induced MD-2/TLR4 association. HK-2 cells were treated with 10 μg/ml LPS for 2 hours in the presence or absence of 2 μM paclitaxel to collect lysate for immunoprecipitation using anti-TLR4 antibodies. The immunoprecipitates were then subjected to immunoblot analysis of TLR4 and MD-2. (B) Densitometric analysis of the MD-2/TLR4 ratio in the immunoblots of the immunoprecipitates. $^\triangle P < 0.05$ versus the mock group or paclitaxel-only group; $^\# P < 0.05$ versus the LPS group. The results show that LPS induced MD-2/TLR4 association and this molecular association was suppressed by paclitaxel. (C) In vitro assay of paclitaxel binding of MD-2. Antibody-sandwich ELISA was conducted as described in the *Materials and Methods*. Decreases in absorbance at 420 nm indicate the binding of paclitaxel of recombinant MD-2. $^* P < 0.05$ versus the 0 μM paclitaxel group; $^\& P < 0.05$ versus the 1 μM paclitaxel group.

leak in vivo (Mirzapoiazova et al., 2007). Interestingly, Lee and colleagues reported that paclitaxel, like LPS, is able to stimulate the translocation of the 50- and 65-kDa heterodimers of NF-κB to the nucleus in 70Z/3 pre-B cells; however, this effect is dose dependent and requires at least 5 μM of paclitaxel (Lee and Jeon, 2001), which is significantly higher than the effective dosage in our study. Furthermore, the stimulatory effect on NF-κB nuclear translocation was shown
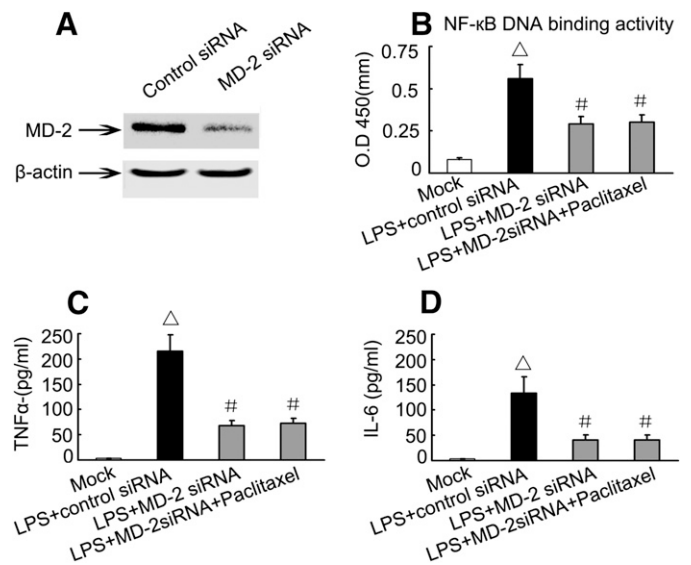
may be related to the different models and doses of paclitaxel used. For example, the chemotherapy dose of paclitaxel (30 μM) used in breast cancer patients is significantly higher than those used in our study (2 μM) and in the study by Mirzapoiazova et al. (10 μM). In addition to the proinflammatory cytokines, it would be interesting to test whether paclitaxel affects the expression of anti-inflammatory cytokines such as IL-10.

Mechanistically, our results suggest that paclitaxel may suppress TLR4-mediated NF-κB signaling by binding MD-2 to inhibit cytokine production in renal tubular cells. Recent studies indicate that microtubule stabilization by paclitaxel may interfere with the effects of LPS on cell–cell junctions, since microtubules and adherence junctions interact dynamically (Bogdan and Ding, 1992; Beutler and Kruys, 1995; Chausovsky et al., 2000). Furthermore, paclitaxel may suppress LPS-induced inflammation by attenuating vascular

Fig. 6. Effect of MD-2 knockdown on LPS-induced NF-κB DNA-binding activity and expression of TNF-α and IL-6 levels. HK-2 cells were transfected with MD-2 siRNA or control siRNA, followed by treatment with 10 μg/ml LPS in the presence or absence of 2μM paclitaxel. (A) Immunoblot analysis to verify MD-2 knockdown by a specific siRNA. (B) NF-κB DNA-binding activity determined using ELISA assay. (C and D) TNF-α and IL-1 expression analyzed by ELISA assay. Data are presented as mean ± S.E.M. ($n = 6$). $^\triangle P < 0.05$ versus the mock group; $^\# P < 0.05$, versus the LPS plus control siRNA group.

after pre-exposing the cells to paclitaxel (Lee and Jeon, 2001). On the other hand, several recent studies have suggested that LPS and paclitaxel share a common receptor/signaling complex (Byrd et al., 1999; Kawasaki et al., 2000; Lee et al., 2000). In line with these studies, our study provides evidence that at low doses, paclitaxel suppresses NF-κB signaling in LPS-induced injury in mouse kidneys and in renal tubular HK-2 cells. We showed that paclitaxel blocks the DNA-binding activity of NF-κB, which is accompanied by the inhibition of cytokine production during LPS treatment. To investigate the underlying mechanisms, we showed that paclitaxel inhibits the association between MD-2 and TLR4 during LPS treatment in HK-2 cells. MD-2 is an extracellular molecule, which, after LPS binding, associates with TLR4 via a specific region to form a complex to trigger downstream signaling (Shimazu et al., 1999; Kawasaki et al., 2001). In our study, MD-2 knockdown suppressed LPS-induced signaling and cytokine production in HK-2 cells. Notably, the anti-inflammatory effect of paclitaxel was attenuated in MD-2 knockdown cells, indicating that paclitaxel functions through MD-2. Using an in vitro binding assay, we further verified that paclitaxel can directly bind to recombinant MD-2, suggesting that paclitaxel may bind to MD-2 to interrupt the MD-3/TLR4 association during LPS treatment. The blockade of MD-2/TLR4 association by paclitaxel explains the observed effects of paclitaxel on downstream proinflammatory responses, including the inhibition of NF-κB activation and suppression of cytokine production. Our observations are consistent with a previous work suggesting that paclitaxel acts through TLR4/MD2 (Kawasaki et al., 2000). According to these results, paclitaxel acts extracellularly by binding to MD-2 to modulate TLR4 signaling. However, paclitaxel is also known to stabilize microtubules to exert related cell biologic effects intracellularly.

In conclusion, our study demonstrates that low doses of paclitaxel can protect against LPS-induced proinflammatory response and kidney injury, suggesting a potential use of the chemotherapy drug in the treatment of septic AKI. Mechanistically, paclitaxel may directly bind MD-2 to block MD-2/TLR4 association, resulting in the inhibition of NF-κB signaling and consequent production of cytokines.

Authorship Contributions

Participated in research design: Zhang.
Conducted experiments: Zhang, Li, Liu.
Performed data analysis: Zhang, Xiang, Li, Dong.
Wrote or contributed to the writing of the manuscript: Zhang, Dong.

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