Abstract. The major role of inner medullary collecting duct (IMCD) cells is to maintain water and sodium homeostasis. In addition to the major role, it also participates in the protection of renal and systemic inflammation. Although IMCD cells could take part in renal and systemic inflammation, investigations on renal inflammation in IMCD cells have rarely been reported. Although berberine (BBR) has been reported to show diverse pharmacological effects, its anti-inflammatory and protective effects on IMCD cells have not been studied. Therefore, in the present study, we examined the anti-inflammatory and protective effects of BBR in mouse IMCD-3 (mIMCD-3) cells against lipopolysaccharide (LPS). An MTT assay was carried out to investigate the toxicity of BBR on mIMCD-3 cells. Reverse transcription quantitative-PCR and western blotting were performed to analyze pro-inflammatory molecules and cytokines. Mechanisms of BBR were examined by western blotting and immunocytochemistry. According to previous studies, pro-inflammatory molecules, such as inducible nitric oxide synthase and cyclooxygenase-2, and pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α are increased in LPS-exposed mIMCD-3 cells. However, the production of these pro-inflammatory molecules is significantly inhibited by treatment with BBR. In addition, BBR inhibited translocation of nuclear factor (NF)-κB p65 from the cytosol to the nucleus, and degradation of inhibitory κ-Bα in LPS-exposed mIMCD-3 cells. In conclusion, BBR could inhibit renal inflammatory responses via inhibition of NF-κB signaling and ultimately contribute to amelioration of renal injury during systemic inflammation.

Introduction

Inner medullary collecting duct (IMCD) is the last part of the collecting duct system, which connects the nephrons to the ureter (1). The main function of IMCD is maintenance of body water homeostasis through regulation of electrolyte and fluid balance by managing the reabsorption and excretion of sodium ions (Na+) and water along with hormonal regulation (2-4). However surprisingly, IMCD also plays a pivotal role in inflammatory responses of the kidney, because it is a preferred site for Escherichia coli adherence (5). Previous studies have reported that pro-inflammatory mediators such as inducible nitric oxide synthase and cyclooxygenase-2, and pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α are increased in LPS-exposed mIMCD-3 cells. However, the production of these pro-inflammatory molecules is significantly inhibited by treatment with BBR. In addition, BBR inhibited translocation of nuclear factor (NF)-κB p65 from the cytosol to the nucleus, and degradation of inhibitory κ-Bα in LPS-exposed mIMCD-3 cells. In conclusion, BBR could inhibit renal inflammatory responses via inhibition of NF-κB signaling and ultimately contribute to amelioration of renal injury during systemic inflammation.
LPS-induced inflammation in rat glomerular mesangial cells and LPS-induced oxidative stress in rat kidneys (19,20). However, the beneficial properties of BBR in LPS-induced inflammatory responses in mouse IMCD-3 cells have not been reported.

Therefore, we firstly examined the potential effects of BBR on LPS-treated mIMCD-3 cells. So, we investigated the production of inflammatory mediators such as iNOS, COX-2, IL-1β, IL-6, and TNF-α in LPS-exposed IMCD cells. Secondly, we examined the regulating mechanisms of BBR including NF-κB and MAPKs against LPS in mIMCD-3 cells.

Materials and methods

Chemicals and reagents. Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F-12 (DMEM/F-12) (cat. no. 11330-032), fetal bovine serum (FBS) (cat. no. 2640-079) and antibiotics (cat. no. 15140122) were obtained from Gibco; Thermo Fisher Scientific, Inc. N-acetylcysteine (NAC) (cat. no. A7250), Bay 11-7082 (cat. no. B5556), Parthenolide (cat. no. P0667) BBR (cat. no. B3412; Purity: 99%), LPS from Lipopolysaccharides (cat. no. L2880; serotype 055:B5) and n-acetylcysteine (NAC) (cat. no. A7250), Bay 11-7082 (cat. no. B5556), Parthenolide (cat. no. P0667) BBR (cat. no. B3412; Purity: 99%), LPS from Lipopolysaccharides (cat. no. L2880; serotype 055:B5) and Parthenolide (cat. no. P0667) were purchased from Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldrich; Sigma-Aldr
three times with PBST, each blot was incubated with horse-radish peroxidase (HRP)-conjugated goat anti-rabbit IgG or donkey anti-goat IgG secondary antibody for 1 h at RT. The proteins were visualized using an enhanced chemiluminescence detection system (cat. no. RPN2232; Amersham; GE Healthcare). Captures protein bands and quantitative analysis were performed using Quantity One® software version 4.6.6 (Bio-Rad Laboratories, Inc.).

**ELISA.** ELISAs for IL-1β, IL-6, and TNF-α were carried out in duplicates in 96-well plates coated with anti-mouse IL-1β, IL-6, and TNF-α monoclonal antibodies in PBS (pH 7.4) at 4°C overnight. The plates were washed with PBST and blocked with PBS containing 10% FBS for 2 h at RT. After two times washes, standards and samples were added and incubated for 2 h at RT, and then the wells were washed and biotinylated anti-mouse IL-1β, IL-6, or TNF-α were added and incubated for 1 h at RT. The wells were washed, avidin-peroxidase was added, and plates were incubated for 30 min at RT. The wells washing again and followed by the addition of TMB substrate. Color development was measured at 450 nm using an automated microplate ELISA reader. Standard samples were run on each assay plate by using serial dilutions of recombinant IL-1β, IL-6, and TNF-α.

**Immunocytochemistry.** mIMCD-3 cells were plated in a chamber slide and incubated with LPS (5 µg/ml) for 30 min at 37°C. The cells were treated with BBR (1 µM) for 1 h before LPS treatment. The cells were fixed in 4% paraformaldehyde for 15 min at RT and washed 3 times with PBS. The cells were treated with 0.1% TritonX-100 for 15 min at RT. After washing, non-specific binding sites were blocked with serum (3% BSA) for 1 h at RT, and incubated with NF-κB antibody (1:250) at 4°C overnight. The cells were then washed and incubated with AlexaFluor®680 goat anti-rabbit IgG (1:2,000; cat. no. A-11011) for NF-κB antibody for 2 h at RT in a darkened room. For nuclear staining, the cells were incubated with DAPI (cat. no. H-1200; Vector Laboratories) at 5 µg/ml for 5 min at RT. The slide was finally washed and mounted for microscopic examination. Stained sections were visualized using a confocal laser microscope (Olympus).

**Nuclear extraction.** mIMCD-3 cells were plated in 100-mm dishes (1×10^5 cells/dish). Then, LPS (5 µg/ml) treated for 0, 15, 30 and 60 min with or without pretreatment of BBR (1 µM) for 1 h. Nuclear extraction assay performed according to the manufacturer's instructions. Briefly, cells were harvested with cell scraper and washed with PBS by centrifugation at 250 x g for 5 min. Add 1X Cytoplasmic Lysis Buffer (containing DTT and Protease inhibitor cocktail) to the pellet and incubation on ice for 15 min. Centrifuge at 250 x g for 5 min, discard the supernatant and add 1X Cytoplasmic Lysis Buffer. Five times drawing and ejecting by 27-gauge syringe, then centrifuge at 8,000 x g for 20 min. Discard supernatant, and add Nuclear Extraction Buffer (containing DTT and Protease inhibitor cocktail). Five times drawing and ejecting by 27-gauge syringe, then gently agitate on rotator at 4°C for 60 min. Centrifuge at 16,000 x g for 5 min, then supernatant was used for experiments.

**NF-xB p65 activation assay.** NF-κB activation was determined in the nuclear extracts of mIMCD-3 cells, following the manufacturer's instructions. Briefly, 15 µg nuclear extract was added to a biotinylated oligonucleotide containing the NF-κB consensus site attached to the streptavidin-coated 96-well plates with agitation on rocking platform at 100 rpm for 1 h. Plates were washed with wash buffer to remove all the unbound reagents. NF-κB p65 primary antibody (1:1,000) was added for 1 h at room temperature without agitation, followed by a goat anti-rabbit secondary antibody conjugated with horseradish peroxidase (1:1,000) at room temperature without agitation. Subsequent to an incubation for 1 h at room temperature, 100 µl developing solution was added to all wells for 3 min at room temperature protected from direct light. The blue color development in the sample wells was monitored until it turned medium to dark blue. Subsequently, 100 µl stop solution was added and the blue color turned yellow. Finally, the absorbance value was ascertained using a spectrophotometer at a wavelength of 450 nm. As a positive control for NF-κB p65 activation, nuclear extracts from Jurkat cells, provided with the kit, were used.

**Statistical analysis.** Results are expressed as the mean ± standard error of the mean (SEM). Statistical significance of intergroup differences was evaluated using two-way ANOVA, with time and dose as variables, followed by a Duncan's post-hoc test. All statistical analyses were performed using SPSS. P<0.05 was considered to indicate a statistically significant difference. All experiments were carried out in triplicates.

**Results**

**Effects of BBR on production of iNOS and COX-2.** Before studying the biological activity of BBR, we performed the MTT assay to evaluate the cytotoxicity of BBR in mIMCD-3 cells. As shown in Fig. 1A, up to a dose of 1 µM, BBR did not affect cell viability. Thus, we chose BBR concentrations of 0.1, 0.5 and 1 µM for further experiments.

To determine the pro-inflammatory effects of BBR against LPS in mIMCD-3 cells, production of pro-inflammatory molecules such as iNOS and COX-2 after LPS stimulation in mIMCD-3 cells was investigated. In accordance with our previous report (2), production of iNOS and COX-2 was elevated in LPS-exposed mIMCD-3 cells. However, the elevation of iNOS and COX-2 was significantly inhibited by BBR in these cells (Fig. 1B-E).

**Effects of BBR on production of pro-inflammatory cytokines.** IL-1β, IL-6, and TNF-α are the representative pro-inflammatory cytokines known to be induced by LPS treatment in mIMCD-3 cells (2). Thus, to measure the effects of BBR on the production of these pro-inflammatory cytokines in LPS-exposed mIMCD-3 cells, we investigated the mRNA and protein expression of IL-1β, IL-6, and TNF-α after LPS stimulation. As reported earlier (2), LPS treatment increased the mRNA and protein levels of IL-1β, IL-6, and TNF-α in mIMCD-3 cells. However, BBR treatment inhibited the induction of IL-1β, IL-6, and TNF-α mRNA and protein levels in a dose-dependent manner (Fig. 2).
Effects of BBR on activation of NF-κB and MAPKs. Since BBR was effective in inhibiting pro-inflammatory mediators in LPS-treated mIMCD-3 cells, we sought to investigate the mechanistic details of the beneficial activity of BBR. Firstly, to rule out the possibility that BBR directly regulates LPS or TLR4, we examined the effect of BBR on TLR4/Md2 complex expression. The methods of Limulus amebocyte lysate (LAL) assay and RT-qPCR are described in Data S1. As shown in Fig. 3A and Fig. S1, BBR did not directly affect LPS and TLR4 expression in mIMCD-3 cells, which means BBR regulates the down-streams upon Tlr4-LPS interaction. Thus, we ought to examine the activation of NF-κB and MAPKs, because activation of both signaling pathways produce pro-inflammatory mediators (22). As shown in Fig. 3B-E, LPS stimulation triggered IκBα degradation, NF-κB p65 translocation from cytosol to nucleus, elevation of NF-κB p65 binding activity, and MAPK phosphorylation. However, treatment with BBR inhibited IκBα degradation, NF-κB p65 translocation and NF-κB p65 binding activity elevation but not MAPK phosphorylation.

Effects of NF-κB on production of inflammatory mediators. To evaluate whether NF-κB deactivation could contribute to amelioration of inflammatory responses in LPS-treated mIMCD-3 cells, we used three well known NF-κB inhibitors (NAC, Bay 11-7082, and Parthenolide). As is known, three inhibitors could block NF-κB p65 translocation from nucleus to cytosol against LPS treatment in mIMCD-3 cells (Fig. S2). Next, we hypothesized that NF-κB inhibitors might downregulate the production of pro-inflammatory mediators, similar to BBR. As expected, NF-κB inhibitors treatment reduced the mRNA levels of iNOS, COX-2, IL-1β, IL-6, and TNF-α after LPS treatment in mIMCD-3 cells to a similar degree as in the cells treated with BBR (Fig. 4).

Discussion

In this study, we revealed that BBR inhibited LPS-mediated inflammatory responses in mIMCD-3 cells. LPS stimulation induced the expression of pro-inflammatory mediators (iNOS and COX-2) and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in mIMCD-3 cells. However, BBR treatment could inhibit these inflammatory mediators and cytokines dramatically. In addition, the mechanism underlying the inhibitory effects of BBR upon LPS stimulation involved the inhibition of NF-κB activation. Our results show that BBR suppresses LPS-mediated inflammation via NF-κB deactivation in mIMCD-3 cells.

Since BBR is so popular with variety biochemical and pharmacological effects and cheap, there are already many ready-to-use drugs associated with BBR. Today, BBR has been developed to dietary supplement, and BBR was approved Unique ingredient identifier (UNII) by FDA (UNII: 0I8Y3P32UF). Based on this information, we can easily examine the beneficial potential of BBR in both non-clinical and clinical experiments. In non-clinical experiments,
Pietra et al. (13) reported that BBR reduce fibrosis and inflammatory cytokine in human dermal fibroblasts in vitro. It was also reported that BBR could protect ulcerative colitis and neuropathic pain in mice (23,24). In addition, many researches have focused on the beneficial effects of BBR in renal diseases. It has been reported that BBR could ameliorate cisplatin-induced nephrotoxicity in mice, that might be through the reduction of histopathological damage, renal functional markers, oxidative stress, cell death signals, and NF-κB activation (25). In addition, BBR have shown to protective and beneficial activities in various renal diseases such as diabetic nephropathy and renal dysfunction (26). Based on many researches about beneficial effects of BBR on renal injury, BBR has been applied to clinical trials in human renal diseases, and has been reported to alleviate the renal injury in type 2 diabetes mellitus (27-29). In addition to previous reports, our results suggest that BBR could be applied in urinary tract infection (UTI), such as cystitis and pyelonephritis. The most common cause of UTI is Escherichia coli (30), and infection spreads from the bladder to the kidneys and collecting systems (31). Therefore, we suggest that BBR inhibits LPS-infected renal inflammation, especially in UTI.

Collecting duct system of the kidney is a renal tubular segment and plays a role in the reabsorption of filtered water (~15%). A key feature of the collecting duct is water reabsorption through hormonal regulation in an autocrine/paracrine manner (32-40). For instance, vasopressin, also called as antidiuretic hormone (ADH), affects osmotic water permeability and increases water reabsorption in IMCD cells (35). Hence, most reports of IMCD have been in the context of regulation of water homeostasis such as osmotic water, urea, and electrolyte permeability (35,41-44). However, because many renal diseases are accompanied by inflammatory mediator production as well as water imbalance (45), it is very important to identify the underlying pathophysiology of inflammatory responses in renal diseases. Thus, regulation of inflammatory mediators could be key in the treatment of renal diseases. Through this study, we propose BBR as a beneficial agent in managing renal inflammation.

Toll-like receptor 4 (TLR4), a transmembrane receptor, belongs to the super-family of pattern recognition receptors (PRRs). Generally, a well-known function of TLR4 is recognition of exogenous molecules from pathogens-associated molecular pattern molecules (PAMPs) and/or damage-associated molecular pattern molecules (DAMPs), which is released upon cellular or tissue damage. TLR4 existed in many cells such as endothelial cells, myocytes, thyroid cells, endometrial cells, mesangial cells, and adipocytes (46-51). We and other researchers identified the expression of TLR4 in IMCD cells (2,5,8). When TLR4 ligand such as LPS
Figure 4. Reduction of pro-inflammatory molecules and cytokine expression by inhibition of the NF-κB pathway in mIMCD-3 cells. mIMCD-3 cells were pre-treated with BBR (1 µM) 1 h before LPS (5 µg/ml) treatment. The expression of TLR4/MD-2 complex was detected after 24 h LPS treatment by flow cytometry. mIMCD-3 cells were incubated with BBR (1 µM) for 1 h. Thereafter, mIMCD-3 cells were harvested after LPS (5 µg/ml) stimulation for 0, 15, 30 or 60 min. Western blotting was performed to demonstrate that BBR could inhibit (B) IκBα degradation and (E) MAPK phosphorylation in mIMCD-3 cells. (C) Immunofluorescence staining was performed to evaluate BBR could inhibit NF-κB translocation from cytosol to nucleus in mIMCD-3 cells. NF-κB p65 was stained with AlexaFluor 568 (red) and Nucleus was stained with DAPI (blue) in mIMCD-3 cells. (D) Binding activity of NF-κB p65 from mIMCD-3 cells was measured using the Trans AM NF-κB p65 assay kit. *P<0.05 vs. saline + LPS 0 min; †P<0.05. Figure shows a representative image from three independent experiments. Scale bar, 10 µm. BBR, berberine; mIMCD-3, mouse IMCD-3; LPS, lipopolysaccharide; IκBα, inhibitory κBα; NF-κB, nuclear factor-κB; TLR4, toll-like receptor 4; NS, not significant.
binds to TLR4, pro-inflammatory mediators such as iNOS, COX-2, IL-1β, IL-6, and TNF-α are up-regulated (5). These inflammatory mediators released from collecting duct cells could trigger renal injury and inflammation, inhibition of inflammatory mediators in collecting duct cells might be important (5). In this study, BBR treatment inhibited TLR4-mediated elevation of inflammatory mediators such as iNOS, COX-2, IL-1β, IL-6, and TNF-α in LPS-treated mIMCD-3 cells, which suggests that BBR attenuated local inflammation in collecting duct cells (Figs. 1 and 2).

In this study, we examined whether activation of NF-κB could regulate the inflammatory mediators. NF-κB belongs to a family of dimeric transcription factors and participates in numerous biological procedures, including immune responses, inflammation, cellular differentiation, proliferation, and survival (52-55). NF-κB activation, followed by LPS treatment, results in induction of cytokines (IL-1, IL-6, and TNF-α) and inflammatory enzymes (iNOS and COX-2) (56-58). Thus, negative regulation of NF-κB might be responsible for inhibition of inflammatory mediators. Our findings showed that BBR treatment inhibited the degradation of Ik-Bα and translocation of NF-κB into the nucleus after LPS treatment, suggesting that BBR inhibited the activation of NF-κB (Fig. 3). In addition, to examine whether NF-κB activation after LPS leads to production of inflammatory mediators in mIMCD-3 cells, we investigated the production of inflammatory mediators after NF-κB inhibition by 3 well-known inhibitor such as NAC (59,60), Bay 11-7082 (61,62), and Parthenolide (62). As expected, the inhibitors showed the inhibitory effects on NF-κB activation in our mIMCD-3 models (Fig. S2). The inhibition of NF-κB by NAC, Bay 11-7082, and Parthenolide significantly inhibited the production of inflammatory mediators (Fig. 4), which suggest that BBR has anti-inflammatory effects mainly through NF-κB deactivation in LPS-exposed mIMCD-3 cells. Some previous researches have reported that BBR inhibits inflammatory mediators and NF-κB signaling in various different disease models (19,63-67). However, there is no report that BBR could have anti-inflammatory and protective effects in LPS-exposed inner medullary collecting duct (IMCD) cells. In this study, we firstly reported that BBR has protective effects upon LPS-induced inflammation by downregulation of NF-κB pathway in mouse IMCD-3 cells.

In summary, we firstly revealed that the anti-inflammatory effects of BBR via deactivation of NF-κB in LPS-stimulated mIMCD-3 cells. Our results suggest that BBR might be a potential and beneficial drug in renal injury. However, the current data are not sufficient to explain the beneficial effects of BBR in in vivo renal injury systems. Therefore, further studies in this area would be needed.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

DGK, JWC and IJJ designed the study, conducted the data analysis and prepared the manuscript. MJK assisted with the experiments. HSL, HJS and SHH analyzed the data and prepared the manuscript. HJS revised the manuscript for intellectual and scientific content. GSB and SJP designed the study and prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References


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