

Research Article

15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ Exerts Antioxidant Effects While Exacerbating Inflammation in Mice Subjected to Ureteral Obstruction

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Urinary obstruction is associated with inflammation and oxidative stress, leading to renal dysfunction. Previous studies have shown that 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) has both antioxidant and anti-inflammatory effects. Using a unilateral ureteral obstruction (UUO) mouse model, we examined the effects of 15d-PGJ₂ on oxidative stress and inflammation in the kidney. Mice were subjected to UUO for 3 days and treated with 15d-PGJ₂. Protein and RNA expression were examined using immunoblotting and qPCR. 15d-PGJ₂ increased NF-E2-related nuclear factor erythroid-2 (Nrf2) protein expression in response to UUO, and heme oxygenase 1 (HO-1), a downstream target of Nrf2, was induced by 15d-PGJ₂. Additionally, 15d-PGJ₂ prevented protein carbonylation, a UUO-induced oxidative stress marker. Inflammation, measured by nuclear NF- κ B, F4/80, and MCP-1, was increased in response to UUO and further increased by 15d-PGJ₂. Renal injury was aggravated by 15d-PGJ₂ treatment as measured by kidney injury molecule-1 (KIM-1) and cortical caspase 3 content. No effect of 15d-PGJ₂ was observed on renal function in mice subjected to UUO. This study illustrates differentiated functioning of 15d-PGJ₂ on inflammation and oxidative stress in response to obstructive nephropathy. High concentrations of 15d-PGJ₂ protects against oxidative stress during 3-day UUO in mice; however, it aggravates the associated inflammation.

1. Introduction

Obstructive uropathy is a common cause of acute kidney disease and often results from the formation of kidney stones that blocks the flow of urine from the kidney. Ureteral obstruction is characterized by inflammatory cell infiltration and by the accumulation primarily of macrophages in the obstructed kidney, accompanied with increased apoptosis and oxidative stress [1].

Cyclooxygenase 2 (COX-2) is induced in response to unilateral ureteral obstruction (UUO), and COX-2-derived prostaglandins have been associated with the proinflammatory response, primarily prostaglandin E₂ (PGE₂) and PGI₂, which are increased in the early phase of inflammation [2]. 15-Deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) is derived from prostaglandin D₂ by dehydration. Increased levels of PGD₂

and 15d-PGJ₂ have been identified in the later phase of inflammation; these have been associated with the resolution of inflammation [3, 4]. Increased prostaglandin biosynthesis during both the onset and resolution of inflammation points to a dual role of COX-2 in the inflammatory process. In addition, the administration of a selective COX-2 inhibitor in the late phase of carrageenan-induced pleurisy reduced PGD₂ and 15d-PGJ₂ concentrations and exacerbated the inflammatory response, supporting an anti-inflammatory role of COX-2 [3].

15d-PGJ₂ is also known as a peroxisome proliferator-activated receptor gamma (PPAR γ) agonist, and the 15d-PGJ₂/PPAR γ pathway has generally been associated with anti-inflammatory actions [5]. In a number of studies, 15d-PGJ₂ has been shown to decrease proinflammatory cytokines and reduce organ injury via PPAR γ activation [6–8]. In

TABLE 1: Primers used for qPCR amplification.

Gene/protein (accession number)*	Forward primer (5'-3')	Reverse primer (5'-3')
<i>Mouse</i>		
CRTH2 (NM_009962.3)	GCGCTATCCGACTTGTAGC	TGAGGAAGAAGACCGAGGAA
DP1 (NM_008962.4)	CTTGCGTTTCTGTCTGTGA	ATGGCTGCTCCAGTTTCTTGT
F4/80 (NM_010130.4)	TGCTAGTGGAGGCAGTGATG	CTGTATTCAACCAGCAGCGA
GAPDH (NM_008084.3)	GACGGCCGCATCTTCTTG	GCGCCCAATACGGCCAAATC
Ly6G (NM_148939.2)	ACTCCTGGTCCAGTGCTCAG	GGCCCTCAGACACCTTATGA
MCP-1 (NM_011333.3)	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCTTCTTG
PPAR γ (NM_011146.3)	CCCTGGCAAAGCATTTGTAT	GAAACTGGCACCTTGAAAA
TNF- α (NM_013693.3)	AGGCTGCCCGACTACGT	GACTTTCTCTGGTATGAGATAGCAAA

*GenBank accession numbers.

contrast, it has been shown that under physiological concentrations, 15d-PGJ₂ induced eosinophil migration via PPAR γ , suggesting that PPAR γ might have biphasic regulatory effects on the immune response [9]. 15d-PGJ₂ has further been recognized as a potent agonist of the PGD₂ receptor chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). The CRTH2 receptor has been associated with increased oxidative stress and proinflammatory actions especially in relation to allergic inflammation [10].

Furthermore, 15d-PGJ₂ itself is chemically reactive and might also form adducts with protein thiol groups and thereby modulate the function of proteins directly [11]. Through this mechanism, 15d-PGJ₂ has been shown to inhibit activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) transcription pathway, which is a key regulator of proinflammatory mediators. In addition, 15d-PGJ₂ has been implicated in the activation of the nuclear factor erythroid 2-related factor 2- (Nrf2-) Kelch-like ECH-associated protein 1 (Keap1) signaling pathway, leading to the activation of gene transcription of antioxidant enzymes including heme oxygenase 1 (HO-1) [12].

Given the reports of the dual roles of 15d-PGJ₂ in regulating both inflammation and oxidative stress, we tested the hypothesis that 15d-PGJ₂ might protect against UUO-induced damage by modifying inflammation and oxidative stress.

2. Materials and Methods

2.1. Experimental Animals. All procedures conformed to the Danish National Guidelines for the care and use of experimental animals and to the published guidelines from the USA National Institutes of Health. The animal protocols were approved by the board of the Institute of Clinical Medicine, Aarhus University, corresponding to the licenses for the use of experimental animals issued by the Danish Ministry of Justice.

Studies were performed on adult (10 weeks of age) C57BL/6J mice. Animals had free access to a standard rodent diet (Altromin, Lage, Germany) and tap water. During the experiments, mice were housed in groups of two to three per cage, with a 12:12 h light-dark cycle, a temperature of

21 \pm 2°C, and a humidity of 55 \pm 2%. Animals acclimatized to the cages for 3-4 days before surgery.

2.2. Experimental Design and Surgical Procedures. 15d-PGJ₂ was dissolved in dimethyl sulfoxide (DMSO) and diluted with saline to a 4% DMSO concentration. 15d-PGJ₂ was administered 2 times daily by s.c. injection at low (0.5 mg/kg/day) and high (1 mg/kg/day) doses ($n = 6$). Control groups received vehicle (4% DMSO in saline) ($n = 6$). Treatment was initiated two days prior to surgery and continued throughout the study. At day 3, mice were anesthetized with sevoflurane (3.5% sevoflurane in O₂/N₂O mixture, 1.5 l/min) and placed on a heating pad to maintain an appropriate body temperature during surgery. Through a midline abdominal incision, the left ureter was exposed and occluded with a 6-0 silk suture. UUO was induced for 3 days, where after the mice were sacrificed and the kidneys removed and prepared for quantitative PCR (qPCR) or semiquantitative immunoblotting. Age- and time-matched, sham-operated controls were prepared and observed in parallel with each experimental group ($n = 5$).

2.3. Blood Sampling. Three days after UUO, mice were anesthetized with sevoflurane. At the time of sacrifice, a blood sample was taken from the left ventricle, and plasma analysis of creatinine and urea levels was performed using a Roche Cobas 6000 analyzer (Roche Diagnostics).

2.4. RNA Isolation and qPCR. Total RNA was purified from the kidney cortex with a NucleoSpin RNA II mini kit following the manufacturer's protocol (Macherey-Nagel, Düren, Germany). RNA was quantitated by spectrophotometry and stored at -80°C. cDNA was synthesized from 0.5 μ g purified RNA using the AffinityScript qPCR cDNA synthesis kit (Life Technologies). For qPCR, 100 ng cDNA served as the template for PCR amplification using the SYBR® Green qPCR Master Mix according to the manufacturer's protocol (Life Technologies). mRNA levels were validated by an Aria Mx3000P qPCR System (Agilent Technologies, Santa Clara, CA, USA) with *Gapdh* as a control gene. Primers used for qPCR amplification are specified in Table 1.

2.5. Immunoblotting. Nuclear and cytoplasmic extracts were harvested from renal cortical tissue using a Nuclear Extract

Kit (Active Motif, Carlsbad, CA, USA) according to manufacturer's protocol. The extracts were used for protein concentration determination and immunoblot analysis, as previously described [13]. Protein concentrations of the extracts were determined using a Pierce BCA protein assay kit (Roche). Briefly, equal amounts of proteins were separated on a 12% Criterion TGX Precast Gel (Bio-Rad Laboratories, København, Denmark) followed by transfer to a Hybond ECL nitrocellulose membrane (GE Healthcare, Hatfield, UK). The membrane was blocked in nonfat dry milk and incubated with primary antibodies (described below) overnight at 4°C. Afterwards, the membrane was incubated with a HRP-conjugated secondary antibody (P448, goat anti-rabbit immunoglobulin, Dako A/S, Glostrup, Denmark). Bound antibody was detected using an enhanced chemiluminescence system (Amersham ECL Plus, GE Healthcare) and visualized on the ChemiDoc MP Imaging System (Bio-Rad). All western blots were normalized to total protein, as measured using Stain-Free technology [14]. GAPDH was examined in parallel and shown as an additional control of equal loading.

2.6. Primary Antibodies. Primary antibodies were obtained from the following sources: caspase-3 (Cell Signaling Technology, Danvers, MA, USA, product number 9665, 1:500), GAPDH (Cell Signaling, product number 2118, 1:2000), HSP90 (Stressgen, San Diego, CA, USA, product number AC88, 1:500), histone H3 (Cell Signaling, product number 4499, 1:1000), Lamin B (Santa Cruz, product number sc-6217, 1:500), HO-1 (Enzo, Farmingdale, NY, USA, product number ADI-SPA-896, 1:500), kidney injury molecule-1 (KIM-1) (R&D Systems, Minneapolis, MN, USA, product number AF1817, 1:500), NF- κ B (Cell Signaling, product number 8242, 1:1000), I κ B α (Cell Signaling, product number 9242, 1:1000), Nrf2 (Santa Cruz, product number sc-722, 1:500), and Keap1 (Santa Cruz, product number sc-33569, 1:500).

2.7. Statistics. All data are presented as the means \pm SE. Multiple comparisons between experimental groups were performed using a one-way ANOVA followed by post hoc analysis using Bonferroni's multiple comparisons test. GraphPad Prism software (GraphPad Software, La Jolla, CA, USA) was used for all statistical analysis. *P* values < 0.05 were considered significant.

3. Results

3.1. 15d-PGJ₂ Promotes Nrf2 Activation during UUO. To investigate the cellular localization of proteins of interest, nuclear and cytoplasmic extracts were purified from kidney cortical tissue. In order to detect any contamination of cytoplasmic protein in nuclear fractions and vice versa, we checked the different fractions by western blotting. We used four different antibodies, the anti-H3 and anti-Lamin B to monitor proteins in the nuclear fraction and anti-GAPDH and anti-HSP90 to monitor proteins in the cytoplasmic fraction. As shown in Figure 1(a), we obtained nuclear fractions free of cytoplasmic proteins and vice versa. Our data

demonstrated that UUO tended to decrease nuclear Nrf2 and increase cytosolic Nrf2 and Keap1 protein expression. Additional 15d-PGJ₂ treatment increased both nuclear and cytosolic Nrf2 as well as Keap1 expression, although this was only significant in the cytoplasm (Figures 1(b) and 1(c)). We further investigated the regulation of the Nrf2 target protein HO-1. HO-1 protein expression was increased in response to UUO and further increased in response to 15d-PGJ₂ treatment (Figures 1(b) and 1(c)). Oxidative stress, estimated from the levels of protein carbonyl groups [15], was increased in response to UUO, and this increase was prevented by 15d-PGJ₂ administration (Figure 1(d)).

3.2. 15d-PGJ₂ Promotes NF- κ B Nuclear Translocation during UUO. The activation of NF- κ B was investigated based on cellular localization of the NF- κ B P65 subunit and on the degradation of the NF- κ B inhibitor I κ B α which plays a role for the nuclear translocation and activation of NF- κ B [16]. NF- κ B was induced in both nuclear and cytoplasmic fractions in response to UUO and further increased after 15d-PGJ₂ administration, indicating a proinflammatory effect of 15d-PGJ₂ (Figures 2(a) and 2(b)). However, western blotting of cytoplasmic I κ B α demonstrated that NF- κ B activation induced by both UUO and treatment of 15d-PGJ₂ was not associated with I κ B α degradation (Figure 2(c)). To further evaluate the inflammatory response, immune cell infiltration and inflammatory markers were analyzed. mRNA levels of the macrophage marker F4/80 were increased in response to UUO and were further increased in response to 15d-PGJ₂ (Figure 2(d)). mRNA levels of neutrophil markers *Ly6G* and *Mcp-1* were likewise increased in response to UUO, where 15d-PGJ₂ further increased the *Mcp-1* mRNA expression at a high dose (Figures 2(e) and 2(f)). *Tnf- α* mRNA levels were increased in response to UUO and slightly induced by 15d-PGJ₂ treatment (Figure 2(g)).

3.3. 15d-PGJ₂ Worsens the Renal Injury Following UUO. 15d-PGJ₂ can exert proapoptotic functions through the activation of several proapoptotic mediators including caspase 3 [17, 18]. Caspase 3 was increased in response to UUO and further increased by 15d-PGJ₂ treatment (Figure 3(a)). Furthermore, cleaved caspase 3 protein abundance increased significantly in response to 15d-PGJ₂ (Figure 3(b)). The response of KIM-1, a general injury marker which regulates inflammation [19], to UUO was also analyzed in the cortex, and KIM-1 protein expression was found to be upregulated after UUO and further increased by 15d-PGJ₂ treatment (Figure 3(c)).

3.4. 15d-PGJ₂ Receptors Are Upregulated in Response to UUO. In order to investigate the receptors responsible for the effect of 15d-PGJ₂ during UUO, we investigated the expression of known 15d-PGJ₂ receptors in response to UUO. The mRNA levels of the receptors *Dp1*, *Crth2*, and *Ppar γ* were upregulated in response to UUO, whereas no change in receptor mRNA expression was found in response to 15d-PGJ₂ treatment (Figures 4(a), 4(b), and 4(c)).

3.5. 15d-PGJ₂ Has No Effect on Renal Function in Response to UUO. The effect of 15d-PGJ₂ on renal function following

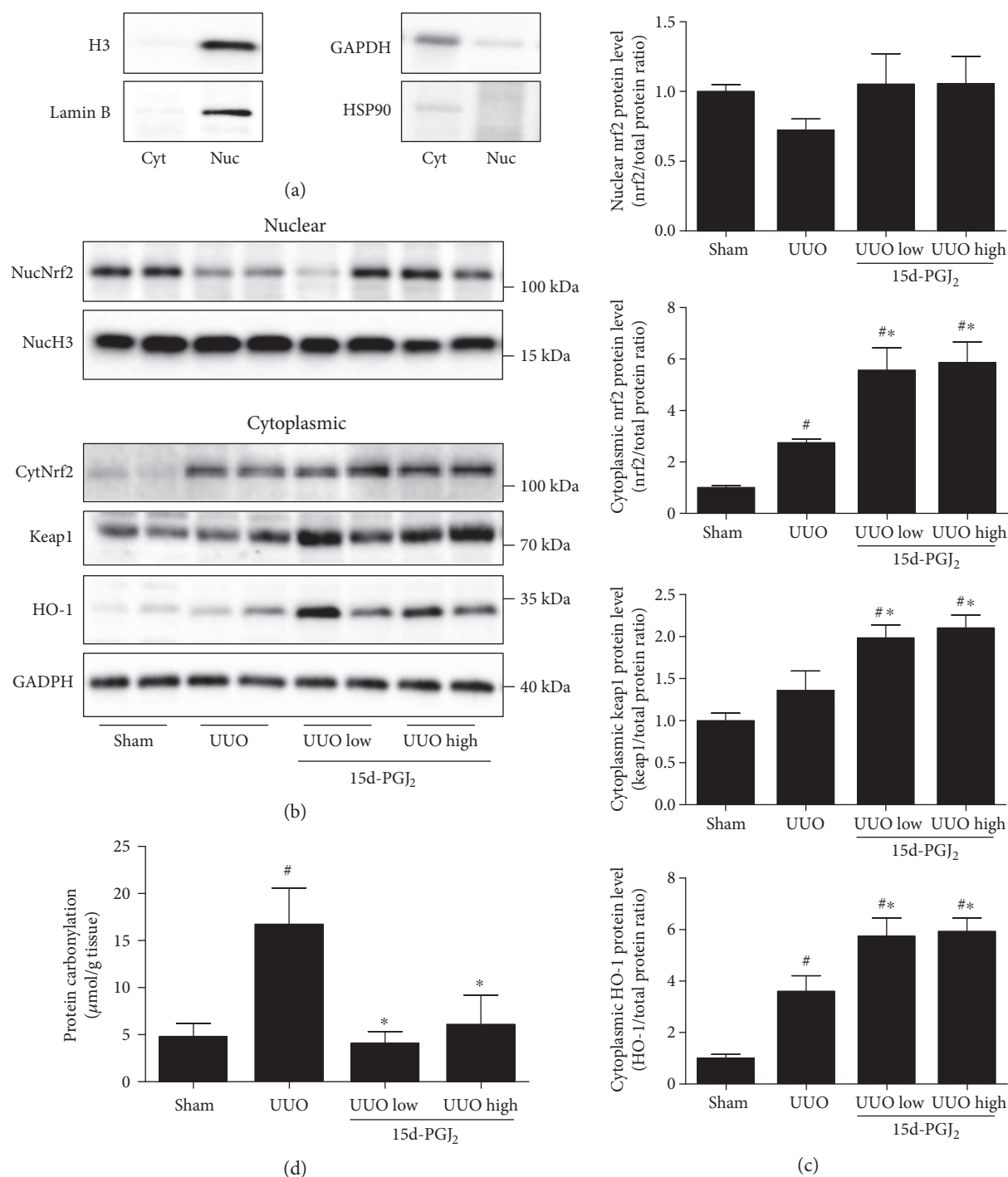


FIGURE 1: Effects of 15d-PGJ₂ on the regulation of Nrf2 localization and antioxidants in the kidney cortex during 3-day UUO. (a) Verification of the purity of nuclear and cytosolic extracts. Nuclear markers, histone H3 and Lamin B, as well as cytosolic markers, HSP90 and GAPDH, were used to show the purity of the tissue extracts. (b-c) Nuclear protein extracts were used to analyze Nrf2 nuclear (Nuc) expression. Cytosolic (Cyt) Nrf2, Keap1, and HO-1 proteins were further analyzed in the cytosolic fraction. Representative western blots are shown. (d) Protein carbonylation. Proteins were extracted from cortical tissue and analyzed using 2,4-dinitrophenylhydrazine to quantitate carbonyl content as a marker of protein oxidation. 15d-PGJ₂ was administered at a low (0.5 mg/kg/day) and a high (1 mg/kg/day) dose as depicted in each graph. Data represents mean \pm SEM. #*P* < 0.05 versus that in sham; **P* < 0.05 versus that in UUO, using ANOVA and Bonferroni's post hoc test (*n* = 5, sham; *n* = 6, UUO groups). Histone H3 (Hist. H3) and GAPDH are shown as loading controls for nuclear and cytoplasmic protein extracts, respectively. UUO, unilateral ureteral obstruction.

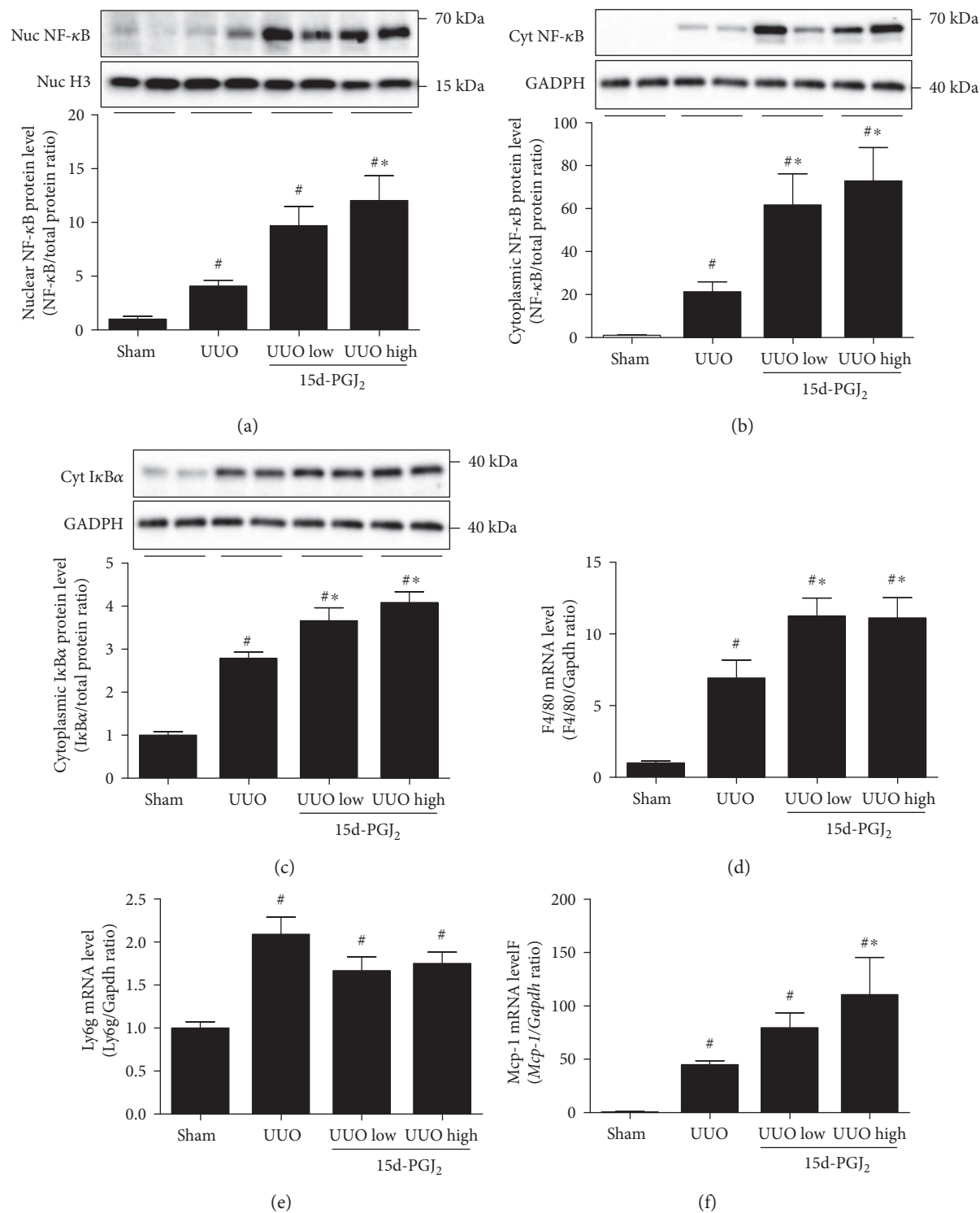


FIGURE 2: Continued.

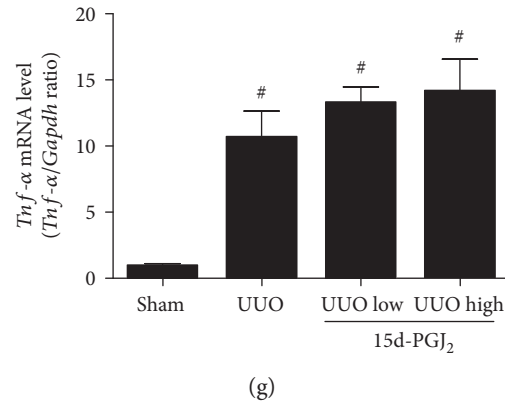


FIGURE 2: 15d-PGJ₂ enhances the proinflammatory response during 3-day UUO (3dUUO). (a) Nuclear protein extracts were used to analyze cortical NF- κ B P65 subunit nuclear protein expression (Nuc). (b) Cortical cytoplasmic protein expression (Cyt) of the NF- κ B P65 subunit. (c) Cortical cytoplasmic protein expression of I κ B α . (d) F4/80 mRNA levels in the kidney cortex. (e) *Ly6G* mRNA levels in the cortex. (f) *Mcp-1* mRNA levels in the cortex. (g) *Tnf-α* mRNA levels in the cortex. Representative blots are shown above each graph. 15d-PGJ₂ was administered at a low (0.5 mg/kg/day) and a high (1 mg/kg/day) dose as depicted in each graph. Data represents mean \pm SEM. # $P < 0.05$ versus that in sham; * $P < 0.05$ versus that in UUO, using ANOVA and Bonferroni's post hoc tests ($n = 5$, sham; $n = 6$, UUO groups). UUO, unilateral ureteral obstruction.

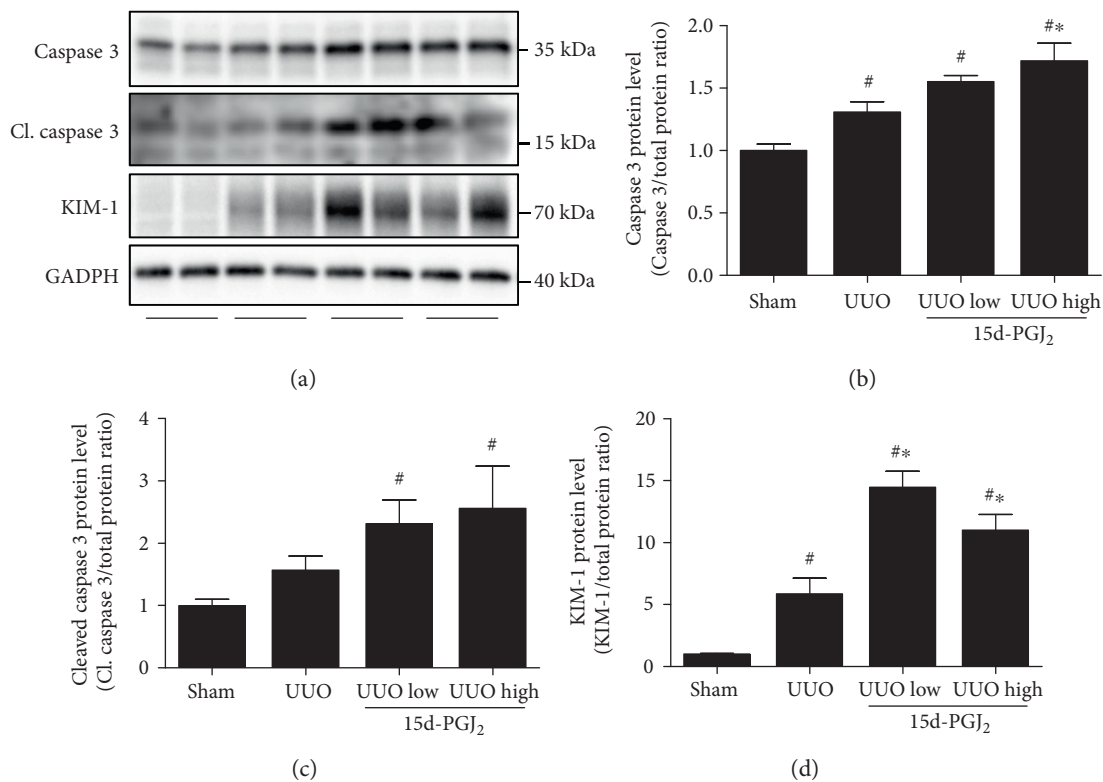


FIGURE 3: Cortical apoptosis and kidney injury are aggravated during UUO by treatment with 15d-PGJ₂. (a) Caspase 3 protein expression. (b) Cleaved caspase 3 (Cl. caspase 3) protein expression. (c) KIM-1 protein expression. Representative blots are shown above each graph. 15d-PGJ₂ was administered at a low (0.5 mg/kg/day) and a high (1 mg/kg/day) dose as depicted in each graph. Data represents mean \pm SEM. # $P < 0.05$ versus that in sham; * $P < 0.05$ versus that in UUO, using ANOVA and Bonferroni's post hoc tests ($n = 5$, sham; $n = 6$, UUO groups).

UUO was evaluated by analyzing kidney weight, plasma creatinine, and BUN (Table 2). 15d-PGJ₂ treatment had no effect on kidney weight and plasma creatinine in UUO mice, but a slight increase in BUN was observed with a high dose of 15d-PGJ₂.

4. Discussion

The main findings of the present study were that high concentrations of 15d-PGJ₂ attenuated oxidative stress and that this was associated with increased HO-1 abundance in the

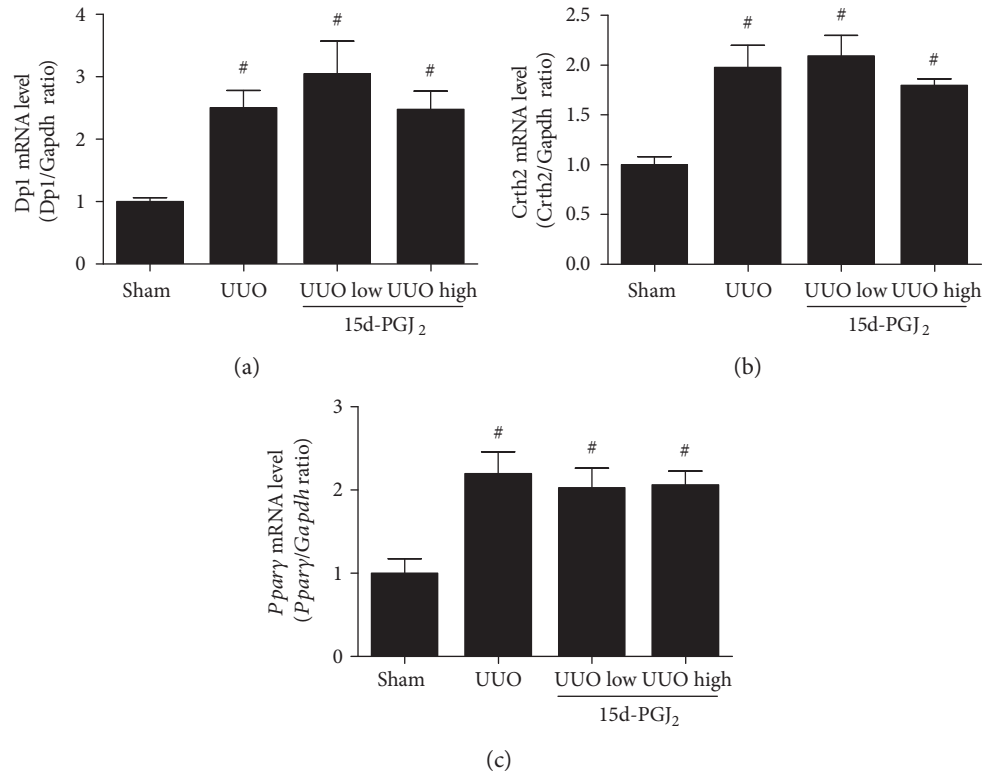


FIGURE 4: 15d-PGJ₂ receptor expression is increased in response to UUO. mRNA was purified from kidney cortical tissue and analyzed using qPCR. (a) *Dp1* mRNA levels in response to UUO. (b) *Crth2* mRNA levels in response to UUO. (c) *Ppary* mRNA levels in response to UUO. 15d-PGJ₂ was administered at a low (0.5 mg/kg/day) and a high (1 mg/kg/day) dose as depicted in each graph. Data represents mean \pm SEM. # $P < 0.05$ versus that in sham, using ANOVA and Bonferroni's post hoc tests ($n = 5$, sham; $n = 6$, 3-day UUO groups). UUO, unilateral ureteral obstruction.

TABLE 2: Effect of 15d-PGJ₂ on body weight, kidney weight, and functional plasma parameters.

	Sham	UUO	UUO + low 15d-PGJ ₂	UUO + high 15d-PGJ ₂
Body weight, g	21.6 \pm 0.8	19.3 \pm 1.0	20.0 \pm 0.5	19.8 \pm 0.5
Obstructed KW, mg/20 g BW	113.3 \pm 4.7	184.8 \pm 3.1 [#]	176.7 \pm 3.1	180.9 \pm 3.2
Plasma creatinine, μ mol/l	7.4 \pm 0.7	9.3 \pm 0.9	10.5 \pm 0.9	8.3 \pm 0.4
BUN, mmol/l	8.9 \pm 0.3	8.6 \pm 0.3	9.1 \pm 0.2	11.2 \pm 0.6*

Biochemical values. Mice were subjected to UUO for 3 days (UUO) and treated with 15d-PGJ₂ at a low dose (0.5 mg/kg/day) and a high dose (1 mg/kg/day). At the time of sacrifice, a blood sample was taken from the left ventricle. Body weight and obstructed kidney weight were measured. Plasma creatinine and BUN were measured using Roche Cobas 6000 analyzer (Roche Diagnostics, Hvidovre, Denmark). Values are means \pm SEM, $n = 6$. # $P < 0.05$ versus that in sham. * $P < 0.05$ versus that in UUO. 15d-PGJ₂: 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂; BW: body weight; KW: kidney weight; BUN: blood urea nitrogen.

obstructed kidney, which might be mediated through increased Nrf2 nuclear translocation. However, the UUO-induced inflammatory response as well as apoptosis was aggravated by high concentrations of 15d-PGJ₂ as demonstrated by increased levels of proinflammatory markers as well as KIM-1 and activation of the caspase 3.

4.1. 15d-PGJ₂ Induces Nrf2 Nuclear Translocation. Oxidative stress is induced in response to UUO, mainly owing to the suppression of antioxidant enzymes [20]. Nrf2 is a transcription factor that regulates cytoprotective proteins including antioxidants such as HO-1 and plays a major role in protection against oxidative damage [21]. Consistent with previous studies [22], we showed that UUO decreases nuclear Nrf2

and increases cytosolic Nrf2 expression resulting in increased oxidative stress. This study supports the role of 15d-PGJ₂ in activation of the Nrf2 pathway and in antioxidant defense against UUO-induced oxidative stress. 15d-PGJ₂ has been shown to activate Nrf2 by covalent modification of the thiol groups of endogenous Keap1, releasing Nrf2 from the Keap1-Nrf2 complex [11]. Thereupon, Nrf2 freely translocates into the nucleus, leading to the transcription of antioxidant genes. However, 15d-PGJ₂ treatment did also increase the renal expression of Keap1 and Nrf2 in the cytoplasm. The reason for this discrepancy remains unclear, but it has previously been suggested that cytosolic Keap1 and Nrf2 protein levels may vary according to experimental models of oxidative stress and the severity of renal injury [23, 24]. One

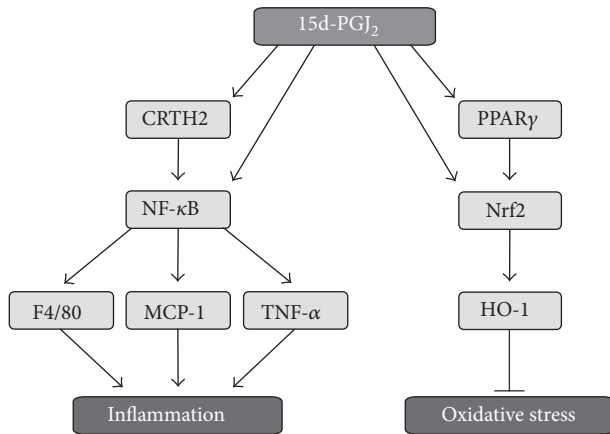


FIGURE 5: Potential mechanisms involved in the antioxidant and proinflammatory actions of 15d-PGJ₂. Ureteral obstruction induces oxidative stress and inflammation. We observed that 15d-PGJ₂ prevents the increase in oxidative stress, probably through activation of the transcription factor Nrf2 and its downstream target HO-1. PPAR γ has previously been shown to activate Nrf2 and might therefore be involved in oxidative protection. 15d-PGJ₂ was shown to exacerbate inflammation through the activation of NF- κ B and its downstream targets MCP-1 and TNF- α as well as via macrophage infiltration (F4/80). CRTH2 has been shown to activate the inflammatory response and might be involved in the 15d-PGJ₂-mediated activation of NF- κ B.

could therefore speculate that other effects of 15d-PGJ₂ or perhaps other pathways can be dominating in this UUO model and play a role in facilitating nuclear translocation rather than directly inducing dissociation of the Nrf2/Keap1 complex. Several in vitro studies have suggested that the protective effects of 15d-PGJ₂ on renal tubular cells might be mediated via the Nrf2 pathway [25–27]. In addition, it has been shown that the 15d-PGJ₂-induced HO-1 levels in serum were absent in Nrf2 KO mice subjected to hepatic ischemia, indicating that the Nrf2 pathway might mediate HO-1 induction in response to 15d-PGJ₂ treatment [28]. However, HO-1 expression was also induced in nontreated UUO mice, so we cannot exclude the possibility that other factors might be involved in the induction of HO-1. The previous studies have demonstrated that HO-1 expression can be induced by other stimuli such as cytokines, NO, and growth factors, and several of these factors are increased in response to renal injury [29]. In addition, our data showed that increased protein carbonylation, a marker of oxidative stress, in response to UUO was prevented by 15d-PGJ₂, further supporting that 15d-PGJ₂ has antioxidant effects on mice subjected to UUO.

4.2. Enhanced NF- κ B Nuclear Translocation in Response to 15d-PGJ₂. Inflammation also plays a major role in the progression of obstructive nephropathy, and the NF- κ B pathway is known to be activated during UUO [30]. 15d-PGJ₂ has been described as an anti-inflammatory prostaglandin in part owing to its ability to form adduct with the I κ B kinase (IKK) to prevent phosphorylation and degradation of I κ B α which binds to the NF- κ B p65 subunit and inhibits NF- κ B nuclear translocation [31], and the activation of NF-

κ B in response to renal I/R was shown to be attenuated by 15d-PGJ₂ administration in rats [32]. In the present study, we observe increased cytosolic NF- κ B protein levels and reduced degradation of I κ B α after 15d-PGJ₂ administration suggesting that 15d-PGJ₂ prevents translocation of NF- κ B from the cytoplasm indicating that 15d-PGJ₂ might have anti-inflammatory properties. In contrast, our data showed that 15d-PGJ₂ treatment can also induce nuclear NF- κ B expression in response to UUO, which argues against an anti-inflammatory function. Although I κ B α is not degraded, I κ B α is most likely modified in some way, which can cause I κ B α to dissociate from NF- κ B, exposing a nuclear localization signal as a prerequisite to nuclear translocation [33]. This result was further supported by the observation of increased macrophage infiltration and *Mcp-1* expression in the kidneys of UUO mice. In addition, it had previously been suggested that KIM-1 can increase *Mcp-1* expression and regulate the immune response of inflammatory cells [19]. So we cannot rule out the possibility that the 15d-PGJ₂-induced KIM-1 expression in UUO mice might also play a role in increasing the expression of *Mcp-1* and infiltration of macrophages.

In vitro studies on murine macrophages have shown that 15d-PGJ₂ retains NF- κ B in the cytoplasm during LPS stimulation [34]. Furthermore, previous studies have demonstrated that 15d-PGJ₂ might induce NF- κ B activity at low levels but directly inhibit NF- κ B at higher levels [35]. Therefore, the pro- and anti-inflammatory functions of 15d-PGJ₂ might be concentration-dependent. This phenomenon could be important for the proinflammatory function of 15d-PGJ₂ we observed during UUO. For example, 15d-PGJ₂ has been shown to induce oxidative stress in LPS-stimulated macrophages, which was associated with increased apoptosis and which might represent a mechanism underlying the 15d-PGJ₂-derived resolution of inflammation [36]. However, most studies investigating apoptosis in response to 15d-PGJ₂ were required to use very high concentrations of 15d-PGJ₂ (μ M range) in order to affect cell viability in vitro [17, 37].

This study is based on an in vivo model, which is a complex model of obstructive kidney disease. The previous studies showing pro- and anti-inflammatory functions of 15d-PGJ₂ are predominantly in vitro studies, which are not directly comparable to our model. Increased inflammation in response to 15d-PGJ₂ treatment may be concentration-dependent. However, we cannot rule out the possibility that off-target effects might occur due to the high concentration of 15d-PGJ₂ used within this study. In addition, during inflammation, other proinflammatory prostaglandins as PGE₂ and TXA₂ might also be produced in excess after UUO. If the system is oversaturated with proinflammatory mediators, the addition of 15d-PGJ₂ could only offer little or no help resulting in increased inflammation.

15d-PGJ₂ has been identified as an agonist for several receptors including PPAR γ , DP1, and CRTH2. In this study, we showed increased expression of these 15d-PGJ₂ receptors in response to UUO. Previously, 15d-PGJ₂ has been shown to exert anti-inflammatory functions through PPAR γ , for example, by the inactivation of NF- κ B [38]. In contrast,

the activation of CRTH2 plays an important role in allergic inflammation [39]. However, it has recently been demonstrated that administration of a CRTH2 antagonist to mice subjected to UUO suppresses the progression of inflammation and fibrosis [40] indicating that activation of the CRTH2 pathway might likewise play a role in UUO-induced injury. Taking into account that 15d-PGJ₂ might show proinflammatory effects on the obstructed kidney, the role of CRTH2 in relation to UUO-induced injury should be investigated. However, further studies will be needed to clarify the role of each receptor in the 15d-PGJ₂-induced regulation of antioxidant defense and inflammation identified in this study.

In summary, this study demonstrated that high concentration of 15d-PGJ₂ decreased oxidative stress-induced damages in response to UUO through the activation of Nrf2. However, the inflammatory response induced by UUO, estimated from nuclear NF- κ B abundance and macrophage infiltration, was worsened by higher concentrations of 15d-PGJ₂ treatment (Figure 5).

5. Conclusion

Our results indicate that high concentrations of 15d-PGJ₂ may exert both antioxidant and inflammatory actions in mice which have been subjected to UUO for 3 days. Since accumulating evidence points towards a potential therapeutic use of 15d-PGJ₂, our findings exhibit the need for a detailed understanding of its targets and mechanisms of action in characterized settings in order to confirm its beneficial effects.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

- [1] A. Dendooven, D. A. Ishola Jr, T. Q. Nguyen et al., "Oxidative stress in obstructive nephropathy," *International Journal of Experimental Pathology*, vol. 92, no. 3, pp. 202–210, 2011.
- [2] E. Ricciotti and G. A. FitzGerald, "Prostaglandins and inflammation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 5, pp. 986–1000, 2011.
- [3] D. W. Gilroy, P. R. Colville-Nash, D. Willis, J. Chivers, M. J. Paul-Clark, and D. A. Willoughby, "Inducible cyclooxygenase may have anti-inflammatory properties," *Nature Medicine*, vol. 5, no. 6, pp. 698–701, 1999.
- [4] T. Shibata, M. Kondo, T. Osawa, N. Shibata, M. Kobayashi, and K. Uchida, "15-deoxy-delta 12,14-prostaglandin J2. A prostaglandin D2 metabolite generated during inflammatory processes," *The Journal of Biological Chemistry*, vol. 277, no. 12, pp. 10459–10466, 2002.
- [5] B. Zingarelli and J. A. Cook, "Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation," *Shock*, vol. 23, no. 5, pp. 393–399, 2005.
- [6] J. M. Kaplan, J. A. Cook, P. W. Hake, M. O'Connor, T. J. Burroughs, and B. Zingarelli, "15-deoxy-delta(12,14)-prostaglandin J(2) (15D-PGJ(2)), a peroxisome proliferator activated receptor gamma ligand, reduces tissue leukosequestration and mortality in endotoxic shock," *Shock*, vol. 24, no. 1, pp. 59–65, 2005.
- [7] M. Abdelrahman, M. Collin, and C. Thiemermann, "The peroxisome proliferator-activated receptor-gamma ligand 15-deoxydelta12,14 prostaglandin J2 reduces the organ injury in hemorrhagic shock," *Shock*, vol. 22, no. 6, pp. 555–561, 2004.
- [8] M. A. Monroy, K. K. Opperman, M. Pucciarelli, S. Yerrum, D. A. Berg, and J. M. Daly, "The PPARgamma ligand 15d-PGJ2 modulates macrophage activation after injury in a murine trauma model," *Shock*, vol. 28, no. 2, pp. 186–191, 2007.
- [9] Y. Kobayashi, S. Ueki, G. Mahemuti et al., "Physiological levels of 15-deoxy-delta12,14-prostaglandin J2 prime eotaxin-induced chemotaxis on human eosinophils through peroxisome proliferator-activated receptor-gamma ligation," *Journal of Immunology*, vol. 175, no. 9, pp. 5744–5750, 2005.
- [10] R. Schuligoi, E. Sturm, P. Luschning et al., "CRTH2 and D-type prostanoid receptor antagonists as novel therapeutic agents for inflammatory diseases," *Pharmacology*, vol. 85, no. 6, pp. 372–382, 2010.
- [11] E. Kansanen, A. M. Kivela, and A. L. Levonen, "Regulation of Nrf2-dependent gene expression by 15-deoxy-delta12,14-prostaglandin J2," *Free Radical Biology & Medicine*, vol. 47, no. 9, pp. 1310–1317, 2009.
- [12] K. Itoh, M. Mochizuki, Y. Ishii et al., "Transcription factor Nrf2 regulates inflammation by mediating the effect of 15-deoxy-delta(12,14)-prostaglandin j(2)," *Molecular and Cellular Biology*, vol. 24, no. 1, pp. 36–45, 2004.
- [13] L. Nilsson, K. Madsen, S. Krag, J. Frøkiær, B. L. Jensen, and R. Norregaard, "Disruption of cyclooxygenase type 2 exacerbates apoptosis and renal damage during obstructive nephropathy," *American Journal of Physiology. Renal Physiology*, vol. 309, no. 12, pp. 1035–1048, 2015.
- [14] A. Gurtler, N. Kunz, M. Gomolka et al., "Stain-free technology as a normalization tool in western blot analysis," *Analytical Biochemistry*, vol. 433, no. 2, pp. 105–111, 2013.
- [15] I. Dalle-Donne, R. Rossi, D. Giustarini, A. Milzani, and R. Colombo, "Protein carbonyl groups as biomarkers of oxidative stress," *Clinica Chimica Acta*, vol. 329, no. 1–2, pp. 23–38, 2003.
- [16] S. Ghosh and M. Karin, "Missing pieces in the NF-kappaB puzzle," *Cell*, vol. 109, no. 2, Supplement 1, pp. S81–S96, 2002.
- [17] D. S. Kang, C. H. Kwon, J. Y. Park et al., "15-deoxy-delta12,14-prostaglandin J2 induces renal epithelial cell death through NF-kappaB-dependent and MAPK-independent mechanism," *Toxicology and Applied Pharmacology*, vol. 216, no. 3, pp. 426–435, 2006.
- [18] D. R. Lee, C. H. Kwon, J. Y. Park, Y. K. Kim, and J. S. Woo, "15-deoxy-delta(12,14)-prostaglandin J(2) induces mitochondrial-dependent apoptosis through inhibition of PKA/NF-kappaB

- in renal proximal epithelial cells," *Toxicology*, vol. 258, no. 1, pp. 17–24, 2009.
- [19] Q. Lin, Y. Chen, J. Lv et al., "Kidney injury molecule-1 expression in IgA nephropathy and its correlation with hypoxia and tubulointerstitial inflammation," *American Journal of Physiology. Renal Physiology*, vol. 306, no. 8, pp. F885–F895, 2014.
 - [20] M. Kinter, J. T. Wolstenholme, B. A. Thornhill, E. A. Newton, M. L. McCormick, and R. L. Chevalier, "Unilateral ureteral obstruction impairs renal antioxidant enzyme activation during sodium depletion," *Kidney International*, vol. 55, no. 4, pp. 1327–1334, 1999.
 - [21] B. H. Choi, K. S. Kang, and M. K. Kwak, "Effect of redox modulating NRF2 activators on chronic kidney disease," *Molecules*, vol. 19, no. 8, pp. 12727–12759, 2014.
 - [22] S. D. Chung, T. Y. Lai, C. T. Chien, and H. J. Yu, "Activating nrf-2 signaling depresses unilateral ureteral obstruction-evoked mitochondrial stress-related autophagy, apoptosis and pyroptosis in kidney," *PloS One*, vol. 7, no. 10, Article ID e47299, 2012.
 - [23] S. Chung, H. E. Yoon, S. J. Kim et al., "Oleanolic acid attenuates renal fibrosis in mice with unilateral ureteral obstruction via facilitating nuclear translocation of Nrf2," *Nutrition & Metabolism (London)*, vol. 11, no. 1, p. 2, 2014.
 - [24] H. J. Kim and N. D. Vaziri, "Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure," *American Journal of Physiology. Renal Physiology*, vol. 298, no. 3, pp. F662–F671, 2010.
 - [25] X. Zhang, L. Lu, C. Dixon et al., "Stress protein activation by the cyclopentenone prostaglandin 15-deoxy-delta12,14-prostaglandin J2 in human mesangial cells," *Kidney International*, vol. 65, no. 3, pp. 798–810, 2004.
 - [26] Y. J. Zhang, X. Yang, Q. Y. Kong et al., "Effect of 15d-PGJ2 on the expression of CD40 and RANTES induced by IFN-gamma and TNF-alpha on renal tubular epithelial cells (HK-2)," *American Journal of Nephrology*, vol. 26, no. 4, pp. 356–362, 2006.
 - [27] X. Li, H. Kimura, K. Hirota et al., "Hypoxia reduces the expression and anti-inflammatory effects of peroxisome proliferator-activated receptor-gamma in human proximal renal tubular cells," *Nephrology, Dialysis, Transplantation*, vol. 22, no. 4, pp. 1041–1051, 2007.
 - [28] S. Bancos, C. J. Bagloli, I. Rahman, and R. P. Phipps, "Induction of heme oxygenase-1 in normal and malignant B lymphocytes by 15-deoxy-delta(12,14)-prostaglandin J(2) requires Nrf2," *Cellular Immunology*, vol. 262, no. 1, pp. 18–27, 2010.
 - [29] E. M. Sikorski, T. Hock, N. Hill-Kapturczak, and A. Agarwal, "The story so far: molecular regulation of the heme oxygenase-1 gene in renal injury," *American Journal of Physiology. Renal Physiology*, vol. 286, no. 3, pp. F425–F441, 2004.
 - [30] V. Esteban, O. Lorenzo, M. Ruperez et al., "Angiotensin II, via AT1 and AT2 receptors and NF-kappaB pathway, regulates the inflammatory response in unilateral ureteral obstruction," *Journal of the American Society of Nephrology*, vol. 15, no. 6, pp. 1514–1529, 2004.
 - [31] J. U. Scher and M. H. Pillinger, "The anti-inflammatory effects of prostaglandins," *Journal of Investigative Medicine*, vol. 57, no. 6, pp. 703–708, 2009.
 - [32] P. K. Chatterjee, N. S. Patel, S. Cuzzocrea et al., "The cyclopentenone prostaglandin 15-deoxy-delta(12,14)-prostaglandin J2 ameliorates ischemic acute renal failure," *Cardiovascular Research*, vol. 61, no. 3, pp. 630–643, 2004.
 - [33] D. A. Jans and S. Hubner, "Regulation of protein transport to the nucleus: central role of phosphorylation," *Physiological Reviews*, vol. 76, no. 3, pp. 651–685, 1996.
 - [34] D. S. Straus, G. Pascual, M. Li et al., "15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 9, pp. 4844–4849, 2000.
 - [35] A. E. Martinez, F. J. Sanchez-Gomez, B. Diez-Dacal, C. L. Oeste, and D. Perez-Sala, "15-deoxy-delta(12,14)-prostaglandin J2 exerts pro- and anti-inflammatory effects in mesangial cells in a concentration-dependent manner," *Inflammation & Allergy Drug Targets*, vol. 11, no. 1, pp. 58–65, 2012.
 - [36] A. Castrillo, P. G. Traves, P. Martin-Sanz, S. Parkinson, P. J. Parker, and L. Bosca, "Potentiation of protein kinase C zeta activity by 15-deoxy-delta(12,14)-prostaglandin J(2) induces an imbalance between mitogen-activated protein kinases and NF-kappa B that promotes apoptosis in macrophages," *Molecular and Cellular Biology*, vol. 23, no. 4, pp. 1196–1208, 2003.
 - [37] P. Reyes-Martin, M. Alique, T. Parra, J. P. Hornedo, and J. Lucio-Cazana, "Cyclooxygenase-independent inhibition of H2O2-induced cell death by S-ketoprofen in renal cells," *Pharmacological Research*, vol. 55, no. 4, pp. 295–302, 2007.
 - [38] K. Chen, J. Li, J. Wang et al., "15-deoxy- gamma 12,14-prostaglandin J2 reduces liver impairment in a model of ConA-induced acute hepatic inflammation by activation of PPAR gamma and reduction in NF- kappa B activity," *PPAR Research*, vol. 2014, p. 215631, 2014.
 - [39] R. G. Townley and S. Agrawal, "CRTH2 antagonists in the treatment of allergic responses involving TH2 cells, basophils, and eosinophils," *Annals of Allergy, Asthma & Immunology*, vol. 109, no. 6, pp. 365–374, 2012.
 - [40] H. Ito, X. Yan, N. Nagata et al., "PGD2-CRTH2 pathway promotes tubulointerstitial fibrosis," *Journal of the American Society of Nephrology*, vol. 23, no. 11, pp. 1797–1809, 2012.

