

Original Article

Rosiglitazone ameliorates cisplatin-induced renal injury in mice

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Abstract

Background. Inflammatory mechanisms may play an important role in the pathogenesis of cisplatin nephrotoxicity. Agonists of the peroxisome proliferator-activated receptor- γ (PPAR γ), such as rosiglitazone, have been recently demonstrated to regulate inflammation by modulating the production of inflammatory mediators and adhesion molecules. The purpose of this study was to examine the protective effects of rosiglitazone on cisplatin nephrotoxicity and to explore the mechanism of its renoprotection.

Methods. Mice were treated with cisplatin with or without pre-treatment with rosiglitazone. Renal functions, histological findings, aquaporin 2 (AQP2) and adhesion molecule expression, macrophage infiltration and tumour necrosis factor- α (TNF- α) levels were investigated. The effect of rosiglitazone on nuclear factor (NF)- κ B activity and on viability was examined using cultured human kidney (HK-2) cells.

Results. Rosiglitazone significantly decreased both the damage to renal function and histological pathology after cisplatin injection. Pre-treatment with rosiglitazone reduced the systemic levels of TNF- α and down-regulated adhesion molecule expression in addition to the infiltration of inflammatory cells after cisplatin administration. Rosiglitazone restored the decreased AQP2 expression after cisplatin treatment. Pre-treatment with rosiglitazone blocked the phosphorylation of the p65 subunit of NF- κ B in cultured HK-2 cells. Rosiglitazone had a protective effect via a PPAR γ -dependent pathway in cisplatin-treated HK-2 cells.

Conclusion. These results showed that pre-treatment with rosiglitazone attenuates cisplatin-induced renal damage through the suppression of TNF- α overproduction and NF- κ B activation.

Keywords: cisplatin; nuclear factor- κ B; rosiglitazone; tumour necrosis factor- α

Introduction

Cisplatin is one of the most broadly effective chemotherapeutic agents used to treat a wide variety of malignancies. However, cisplatin is a potent toxin to renal tubules and is associated with a cumulative decline in renal function. About 25–35% of the patients administered with cisplatin develop a mild and partially reversible decline in renal function after the first course of therapy [1]. Although, many reports have suggested that cell cycle events and metabolic responses seem to be important determinants of the degree of renal failure induced by cisplatin, recent studies have strongly suggested that inflammatory mechanisms play an important role in the pathogenesis of cisplatin nephrotoxicity [2,3]. Cytokines, particularly tumour necrosis factor- α (TNF- α), appear to contribute to cisplatin-induced renal injury and to coordinate the activation of a large network of chemokines and cytokines in the kidney following cisplatin injection [3]. TNF- α activates nuclear factor (NF)- κ B by triggering a signalling pathway that leads to the phosphorylation and consequent degradation of the inhibitory protein I κ B α . The degradation of I κ B α exposes a nuclear localization signal on the NF- κ B protein, which then moves into the nucleus and stimulates the transcription of specific genes encoding inflammatory mediators [4]. In addition, inhibition of either TNF- α production or its activity reduces cisplatin-induced renal dysfunction and structural damage [5]. Therefore, anti-inflammatory treatment against cisplatin nephrotoxicity may be a novel therapeutic or preventive strategy.

The peroxisome proliferator-activated receptor- γ (PPAR γ) is a member of the nuclear receptor superfamily of ligand-activated transcription factors [6]. Ligand-activated PPAR γ binds to a specific DNA binding site, termed the peroxisome proliferator

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response element, to regulate the transcription of numerous target genes that affect inflammation, such as cytokines and adhesion molecules [7]. Recently, it has been shown that PPAR γ is expressed in human monocyte-derived macrophages and that PPAR γ agonists inhibit monocyte inflammatory cytokine production and macrophage activation [8,9]. It has also been reported that a PPAR γ agonist has a potent anti-inflammatory effect in human endothelial cells [10]. Previous studies have suggested that ligand-activated PPAR γ can down-regulate NF- κ B transcription [11].

Since TNF- α production is under the control of NF- κ B and is associated with the pathogenesis of cisplatin nephrotoxicity, we have investigated whether rosiglitazone ameliorates cisplatin-induced renal dysfunction in mice. Our results reveal that pre-treatment with rosiglitazone attenuates cisplatin-induced renal damage through the suppression of systemic TNF- α overproduction and NF- κ B activation via a PPAR γ -dependent pathway.

Materials and methods

Animals and drug treatment

Male C57BL/6 mice (Charles River Korea, Seoul, Korea) were given a standard laboratory diet and water *ad libitum* for and were cared, under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School. At the start of the experiments, the mice were 8–10 weeks of age, weighing 25–30 g. After a minimum 7-day acclimation period, a group of mice was administered a single intraperitoneal injection of either vehicle (saline) or cisplatin (20 mg/kg BW; Sigma Chemical Co., St Louis, MO, USA). To obtain an optimal concentration of the drug and time, we have performed dose-dependent experiments using the same concentrations and time of treatment *in vivo* (dose of 5, 7.5, 10 or 12.5 mg/kg and treatment time of 1, 2, 3, 5 or 7 days). We have confirmed the data on the anti-inflammatory effect of rosiglitazone at over 10 mg/kg once a day for 3 days. Some groups of mice received rosiglitazone (10 mg/kg, donated by Glaxo SmithKline Pharmaceuticals, Seoul, Republic of Korea) either alone or before cisplatin injection. Rosiglitazone was administered through oral gavage once a day for 3 days, followed by intraperitoneal cisplatin injection. Mice were anaesthetized with pentobarbital (30 mg/kg), subsequently sacrificed at 24, 48 and 72 h after cisplatin injection by cervical dislocation, and then renal tissue and blood were collected.

Biochemical analysis

Each group of mice was housed in a separate metabolic cage to collect urine quantitatively and measure water intake. On the day of sacrifice, blood was collected and osmolality, urea nitrogen and creatinine concentrations were measured in the plasma. Urea nitrogen and creatinine levels in blood and urine were measured using an enzymatic method (SRL, Tokyo, Japan). Urine and plasma osmolalities were measured using an Advanced CRYOMATICTM osmometer

(Model 3C2, Needham Height, MA, USA). Free water reabsorption ($T^C H_2O$) was calculated by the following formula: $T^C H_2O = V \times (U_{osm} / P_{osm} - 1)$, where V is urine volume, U_{osm} is urine osmolality and P_{osm} is plasma osmolality. Serum insulin and C-peptide levels were analysed by using radioimmunoassay at the Neodine Laboratories (Seoul, South Korea).

Histological examination

The mouse kidney was sectioned in blocks and fixed in 10% formalin, then dehydrated in graded concentrations of alcohols, and embedded in paraffin. The kidney block was cut into 5 μ m sections and stained with haematoxylin–eosin. Tubular damage was examined in periodic acid-schiff (PAS) stained sections using the percentage of cortical tubules showing epithelial necrosis: 0 = normal; 1 = <10%; 2 = 10–25%; 3 = 26–75%; 4 = >75%. Apoptosis was assessed by terminal deoxynucleotidyl transferase-mediated uridine triphosphate (dUTP) nick-end labelling (TUNEL) and the number of apoptotic cells, as defined by chromatin condensation or nuclear fragmentation (apoptotic bodies), was counted. Apoptosis was detected in the specimen using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon International, Temecular, CA, USA), according to the manufacturer's protocol. The morphometric examination was performed by two independent, blinded investigators.

Cell culture

The immortalized proximal tubule epithelial cell lines from the normal adult human kidneys (HK-2) or human malignant glioblastoma cell lines (9L-glioma) were purchased from the American Type Cell Collection and cultured as described previously [12]. Cells were passaged every 3–4 days or 2–3 days in 100 mm dishes (Falcon, Bedford, MA, USA) using Dulbecco's modified Eagle's medium (DMEM)-F12 (Sigma Chemical Co.) or DMEM supplemented with 10% fetal bovine serum (FBS; Life Technologies Inc., Gaithersburg, MD, USA), insulin–transferrin–sodium selenite media supplement (Sigma Chemical Co.), 100 units/ml penicillin and 100 g/ml streptomycin (Sigma Chemical Co.). These cells were incubated in a humidified atmosphere of 5% CO₂, 95% air at 37°C for 24 h and were subcultured at 70–80% confluence.

Application of cisplatin, PPAR γ agonists and PPAR γ antagonist in cultured cells

We have performed experiments using concentrations of 1, 5, 10 or 20 μ M and treatment times of 10, 30 or 60 min with rosiglitazone. We obtained significant protective data at a concentration of 5 μ M and at a time of 30 min treatment. On the basis of the results, 5 μ M rosiglitazone and 30 min incubation time were used throughout the study. For experimental use, HK-2 cells or 9L-glioma cells were plated onto 60 mm dishes in a medium containing 10% FBS for 24 h and cells were switched to DMEM-F12 or DMEM with 2% FBS for 16 h. These cells were either treated with cisplatin or not (1 μ g/ml; Sigma Chemical Co.), rosiglitazone (5 μ mol; Cayman Chemicals, Ann Arbor, MI, USA), troglitazone

(5 μ M; Cayman Chemicals), ciglitazone (5 μ M; Cayman Chemicals), 15-deoxy- Δ 12, 14-prostaglandin J₂ (15d-PGJ₂) (5 μ M; Cayman Chemicals) or GW-9662 (2 μ M; Cayman Chemicals). Control cells received buffer only instead of rosiglitazone and/or cisplatin. At the end of the treatment, the cells were harvested.

Measurement of TNF- α level

The level of TNF- α in serum was determined by using enzyme-linked immunosorbent assay (ELISA) kits (Endogen, Woburn, MA, USA), according to the manufacturer's instructions. In all the cases, a standard curve was constructed from standards provided by the manufacturer.

Western blot analysis

Western blot analysis was performed as previously described [13]. Samples were mixed with sample buffer, boiled for 10 min, separated by 12% SDS-polyacrylamide gel electrophoresis under denaturing conditions and electroblotted to nitrocellulose membranes. The nitrocellulose membranes were incubated with a blocking buffer containing 5% non-fat dry milk in Tris-buffered saline Tween-20 (TBST) buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween-20), and incubated with rabbit polyclonal antibody against phospho-p65 (Cell Signaling Technology Inc, Vebery, MA, USA; dilution 1:1000) or rabbit polyclonal antibody against p65 (Upstate, Lake Placid, NY, USA; dilution 1:1000), with anti-inter cellular adhesion molecule (ICAM)-1 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:2000), with affinity-purified anti-PPAR γ polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:2000), or with affinity-purified anti-rabbit polyclonal aquaporin 2 (AQP2) (Alomone Laboratories, Jerusalem, Israel; dilution 1:2000). The blots were washed with phosphate-buffered saline (PBS) and incubated with horseradish peroxidase-conjugated secondary antibody. Signals were visualized by chemiluminescent detection according to the manufacturer's protocol (Amersham Pharmacia Biotech, London, UK). The membranes were reprobed with anti-actin or p65 antibody to verify equal loading of protein in each lane. All signals were visualized and analysed by densitometric scanning (LAS-1000, Fuji Film, Tokyo, Japan).

Immunohistochemical analysis

Isolated kidney tissue was fixed by immersion in 4% paraformaldehyde and blocked in paraffin. Tissue sections were placed on glass slides, deparaffinized with xylene and rehydrated with graded ethanols. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min, and the samples on slides were then rinsed with PBS. To obtain an adequate signal, samples were treated with pepsin at 42°C for 5 min. After treatment with the blocking buffer, the slides were incubated overnight at 4°C with primary antibodies described above for ICAM-1 (Santa Cruz Biotechnology; dilution 1:50), AQP2 (Alomone Laboratories; dilution 1:50), with rabbit polyclonal antibody against phospho-p65 (Santa Cruz Biotechnology; dilution 1:100) or with a rat anti-mouse F4/80 antibody (Serotec Inc., Oxford, UK; dilution 1:50).

The primary antibody was visualized using the Vectastain ABC-Elite peroxidase detection system (Vector Laboratories, Burlingame, CA, USA), followed by reaction with diaminobenzidine as chromogen and counterstaining with haematoxylin (Sigma Chemical Co.). Evaluation of samples was performed by an observer who was unaware of the origin of samples. The number of F4/80-positive cells or NF- κ B-activated cells in each section was calculated by counting the number of positively stained cells in 10 \times 400 fields per slide.

Cell viability assay

Cell survival was evaluated by the microculture tetrazolium assay using sodium 3'-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT) assay (Cell Proliferation Kit II, Roche, Penzberg, Germany) according to the manufacturer's instructions. Cells were seeded in 100 ml, 96-well plates (5000 cells/well). After 24 h, experimental agents were applied (100 ml) and the cells were incubated for 24 h at 37°C. The XTT solution was added to each well (50 μ l). The cells were incubated at 37°C for 8 h, and absorbance at 490 nm was measured with the Bio-Rad model 3550 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). All the experiments were performed in quadruplicate.

Statistical analysis

Data are expressed as mean \pm SEM. Multiple comparisons were examined for significant differences using analysis of variance (ANOVA), followed by individual comparisons with the Tukey post-test. Statistical significance was set at $P < 0.05$.

Results

Effect of rosiglitazone on renal function in cisplatin nephrotoxicity

As shown in Table 1 and Figure 1, cisplatin injection significantly increased blood urea nitrogen (BUN) and serum creatinine levels at 72 h. In contrast, administration of rosiglitazone in the cisplatin-treated group reduced significantly both the BUN and creatinine levels compared with cisplatin alone. The urinary volume increased in association with decreases in its osmolality and free water reabsorption in cisplatin-treated control group, but the values of these parameters were significantly changed in mice pretreated with rosiglitazone.

Effect of rosiglitazone on renal histology in cisplatin nephrotoxicity

Histochemical examination revealed necrosis, protein cast, vacuolation and desquamation of epithelial cells in renal tubules from 48 h after cisplatin treatment. However, pre-treatment with rosiglitazone dramatically improved the cisplatin nephrotoxicity, and less histological damage was observed in renal tubules (Figure 2). Renal tubular epithelial cell apoptosis was

Table 1. Functional parameters at 72 h from control and cisplatin-treated mice with or without the pre-treatment with rosiglitazone

| | Con | Ros | Cis | Cis ± Ros |
|---|-------------|--------------|---------------|--------------------------|
| BUN (mg/dl) | 20 ± 2.0 | 19 ± 1.3 | 161.5 ± 28.4* | 54.3 ± 10.2 [†] |
| Scr (mg/dl) | 0.52 ± 0.1 | 0.53 ± 0.2 | 2.5 ± 0.5* | 1.2 ± 0.2 [†] |
| U _{osm} (mOsm/kg H ₂ O) | 2,148 ± 112 | 2,485 ± 138 | 680 ± 14* | 1,152 ± 105 [†] |
| P _{osm} (mOsm/kg H ₂ O) | 336.0 ± 9.1 | 345.3 ± 12.8 | 388.5 ± 11.3* | 351.8 ± 6.2 [†] |
| (U/P) _{osm} | 6.4 ± 0.5 | 7.2 ± 0.4 | 1.7 ± 0.1* | 3.3 ± 0.3 [†] |
| UV (μl/day) | 280 ± 29 | 289 ± 12 | 530 ± 57* | 478 ± 36 |
| T ^C H ₂ O (μl/min/kg) | 49.7 ± 6.6 | 57.3 ± 1.6 | 25.7 ± 2.7* | 43.3 ± 2.4 [†] |

Values are expressed as mean ± SEM. Con, saline; Ros, rosiglitazone; Cis, cisplatin; Cis ± Ros, cisplatin injection with rosiglitazone pre-treatment; BUN, blood urea nitrogen; Scr, serum creatinine; U_{osm}, urine osmolality; P_{osm}, plasma osmolality; (U/P)_{osm}, urine-to-plasma osmolality ratio; UV, urine volume; T^CH₂O, solute-free water reabsorption; **P* < 0.05 vs saline, [†]*P* < 0.05 vs cisplatin, *n* = 5–8 in each group.

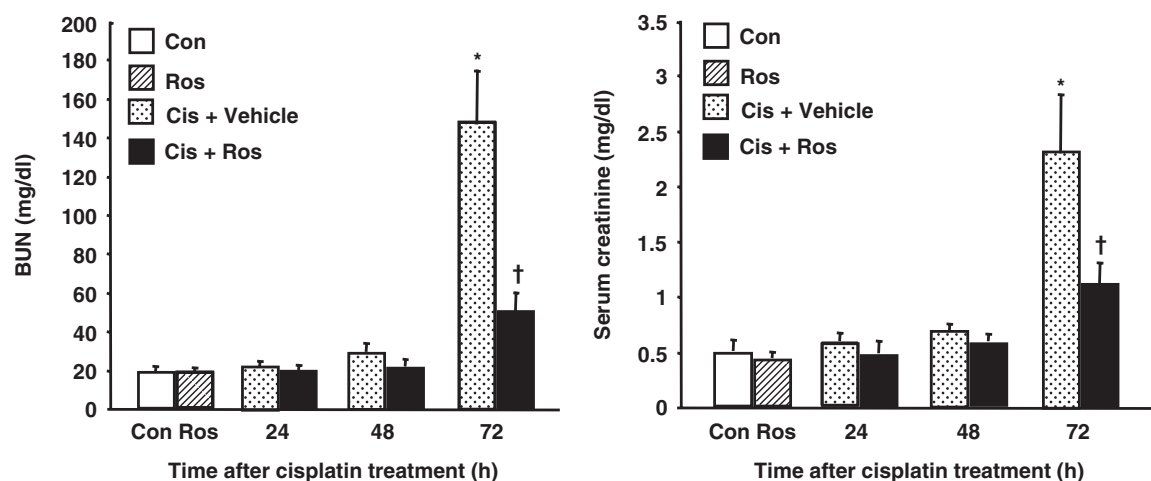


Fig. 1. Effect of rosiglitazone on renal function in cisplatin-treated mice. Values are expressed as mean ± SEM. Con, saline; Ros, rosiglitazone; Cis, cisplatin; Cis ± Ros, cisplatin injection with rosiglitazone pre-treatment; BUN, blood urea nitrogen. **P* < 0.05 vs saline, [†]*P* < 0.05 vs cisplatin, *n* = 5–8 in each group.

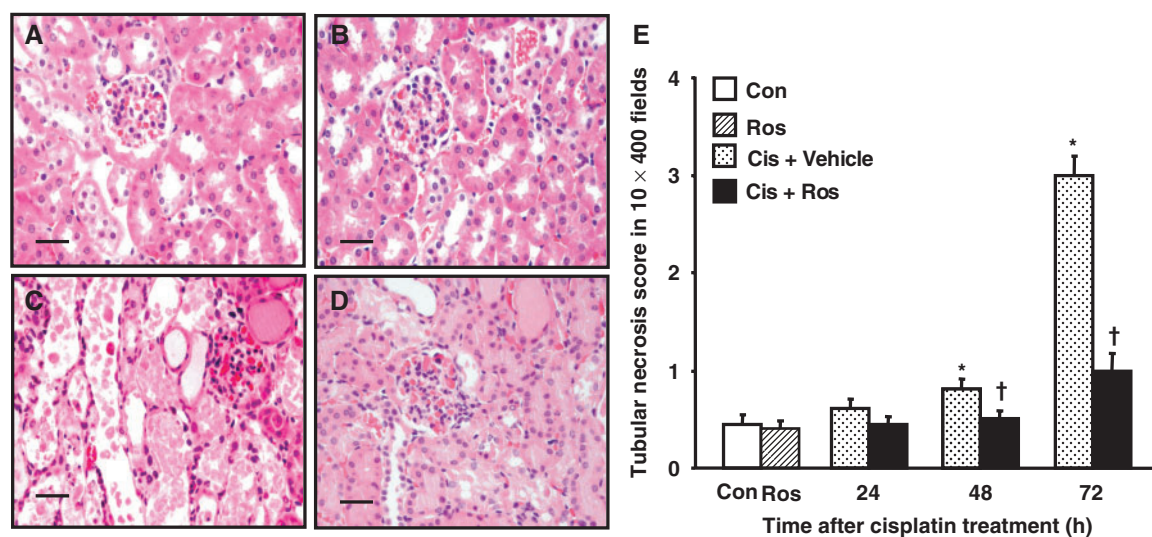


Fig. 2. Effect of rosiglitazone on renal histology in cisplatin-induced renal injury in mice. Renal histological changes at 72 h after cisplatin administration (A–D). Mice were treated with saline (A; Con), rosiglitazone (B; Ros), cisplatin (C), or cisplatin with pre-treatment of rosiglitazone (D). (E) tubular necrosis score. Data represent mean ± SEM from four independent experiments. **P* < 0.05 vs saline; [†]*P* < 0.05 vs cisplatin. Kidneys were harvested at 24, 48 and 72 h after injection. The cisplatin-treated kidneys (C) showed marked injury with cast formation, sloughing of tubular epithelial cells, loss of brush border and dilation of tubules. These changes were less pronounced in rosiglitazone pre-treated mice (D). Rosiglitazone treatment alone (B) had no effect on renal histology. Bars indicate 50 μm. Magnification, 400×.

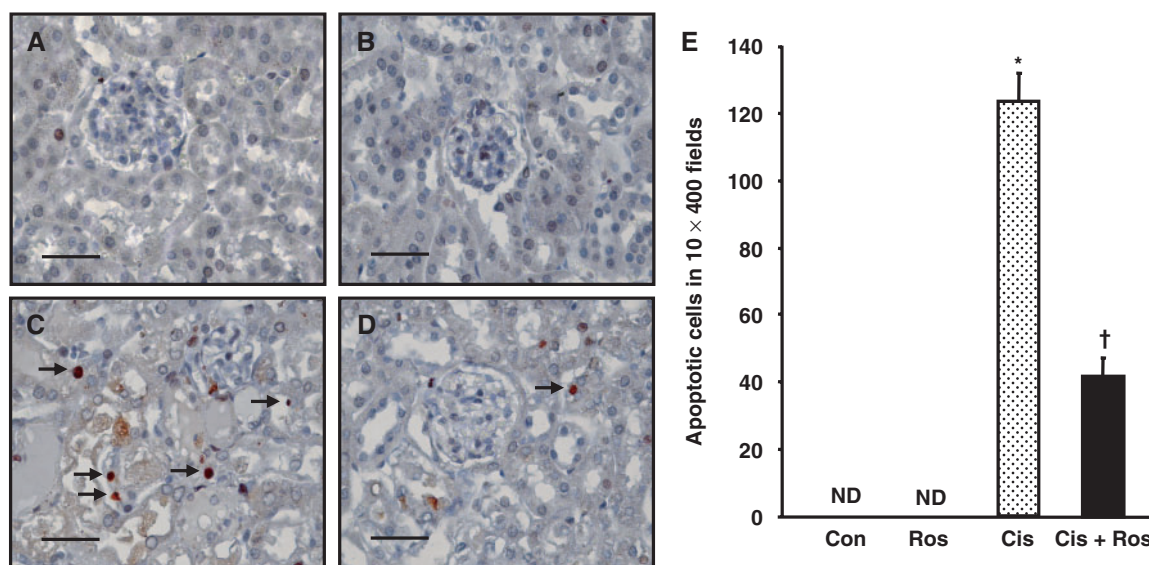


Fig. 3. Effect of rosiglitazone on tubular apoptosis in cisplatin-induced renal injury in mice. Tubular apoptosis was scored in renal tissues at 72 h from mice treated with saline (A; Con), rosiglitazone (B; Ros), cisplatin (C; Cis), or cisplatin with pre-treatment of rosiglitazone (D; Cis ± Ros). Kidneys from cisplatin-treated control group showed nuclear changes consistent with apoptotic cell death. Apoptotic cells were defined by chromatin condensation or nuclear fragmentation (apoptotic bodies). The pre-treatment with rosiglitazone significantly reduced the extent of apoptotic cell death in cisplatin-treated group. Data represent mean ± SEM from four independent experiments. ND, not detected. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin. Bars indicate 50 μ m. Magnification, 400 \times . Arrows indicate TUNEL-positive apoptotic cells.

quantified and confirmed by TUNEL. Kidneys from cisplatin-treated control group showed nuclear changes consistent with apoptotic cell death. The pre-treatment with rosiglitazone significantly reduced the extent of apoptotic cell death in cisplatin-treated group (Figure 3).

Effect of rosiglitazone on AQP2 expression in cisplatin nephrotoxicity

Pre-treatment with rosiglitazone tended to reduce an increase of urinary volume and significantly ameliorated a decrease of urine osmolality and free water reabsorption in the cisplatin-treated mice. A previous report has indicated that cisplatin decreases the abundance of aquaporin water channels, which accounts for the cisplatin-induced urinary concentration defect [14]. Therefore, we examined the expression of AQP2 proteins in the cortex and medulla of the kidney. The anti-AQP2 antibody recognized 29 kDa and 35–50 kDa bands, corresponding to non-glycosylated and glycosylated AQP2. The expression of AQP2 was significantly lower in the cortex and medulla of cisplatin-treated mice than that of the control mice. By pre-treatment with rosiglitazone in cisplatin-treated mice, the AQP2 expression in both cortex and medulla was significantly increased as compared with cisplatin treatment alone (Figure 4A). The immunoreactivity for AQP2 was observed in the principal cells of collecting ducts, both in the apical region of the cell and throughout the cytoplasm, in control and rosiglitazone-alone treatment groups (Figure 4B and C). However, the AQP2 labelling was

decreased by treatment of cisplatin (Figure 4D). The administration of rosiglitazone restored the AQP2 labelling similar to its labelling in normal kidney (Figure 4E).

Effect of rosiglitazone on infiltration of monocyte/macrophages in cisplatin nephrotoxicity

The infiltration of inflammatory cells is a major characteristic in cisplatin nephrotoxicity. We examined the effect of rosiglitazone on the infiltration of cisplatin-induced inflammatory cells. Cisplatin started to increase F4/80 staining at 24 h, and this increase was significantly reduced by pre-treatment with rosiglitazone (Figure 5).

Effect of rosiglitazone on expression of ICAM-1 in cisplatin nephrotoxicity

Adhesion molecules have a role first in the recruitment, and then in the consequent adhesion and transmigration of inflammatory cells. Therefore, we studied the effect of rosiglitazone on the expression of ICAM-1 in cisplatin-induced renal damage. The increased protein level of ICAM-1 was evident at 24 h and persisted 72 h after cisplatin administration. Pre-treatment with rosiglitazone significantly decreased the protein level of ICAM-1 in cisplatin-treated mice when compared with renal tissues obtained from only cisplatin-treated mice (Figure 6A). When compared with renal tissues obtained from vehicle-treated mice, renal tissues obtained from cisplatin-treated mice showed a

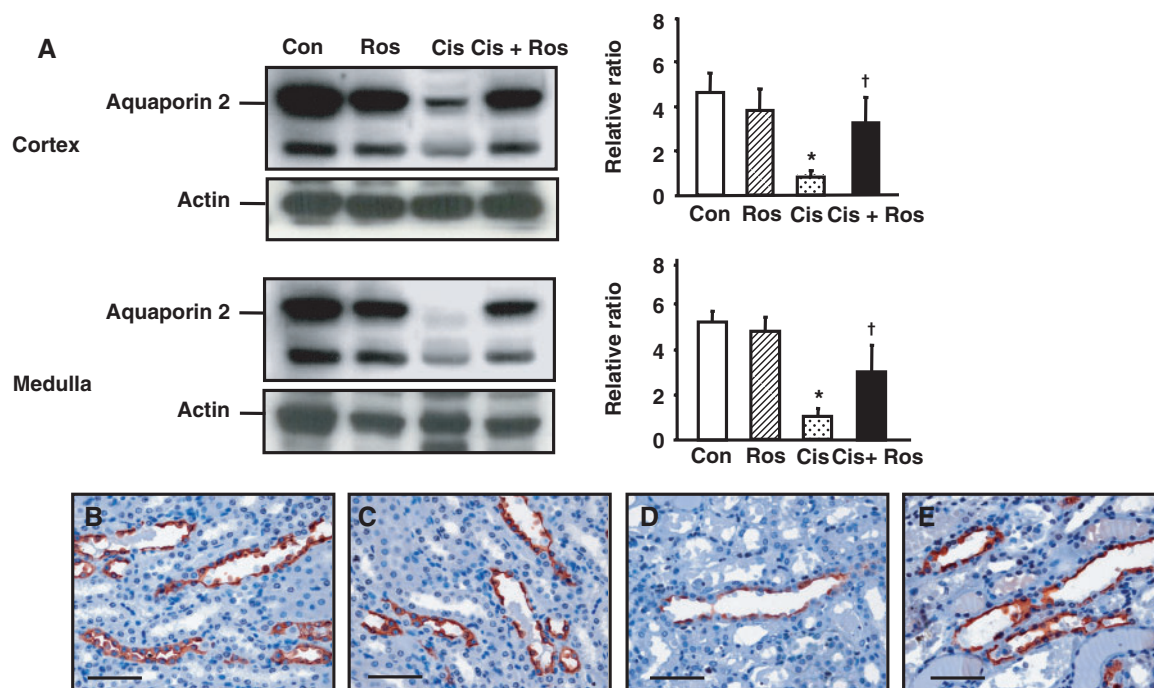


Fig. 4. Effect of rosiglitazone on AQP2 expression in cisplatin-induced renal injury in mice. Western blots and corresponding densitometric analyses of AQP2 expression in renal tissue at 72 h after cisplatin administration (A). Con, saline; Ros, rosiglitazone; Cis, cisplatin; Cis ± Ros, cisplatin injection with rosiglitazone pre-treatment. Data represent mean ± SEM from four independent experiments. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin. (B–E) Immunohistochemical localization of AQP2 in renal tissue at 72 h after cisplatin treatment. Mice were treated with saline (B), rosiglitazone (C), cisplatin (D) or cisplatin with rosiglitazone pre-treatment (E). The abundance of AQP2 expression was seen exclusively in the principal cells of collecting duct, both in the apical region and throughout the cytoplasm (B and C). The expression of AQP2 was reduced by cisplatin (D). Pre-treatment with rosiglitazone restored the expression of AQP2 similar to its expression in normal kidney (E). Bars indicate 50 μ m. Magnification, 400 \times .

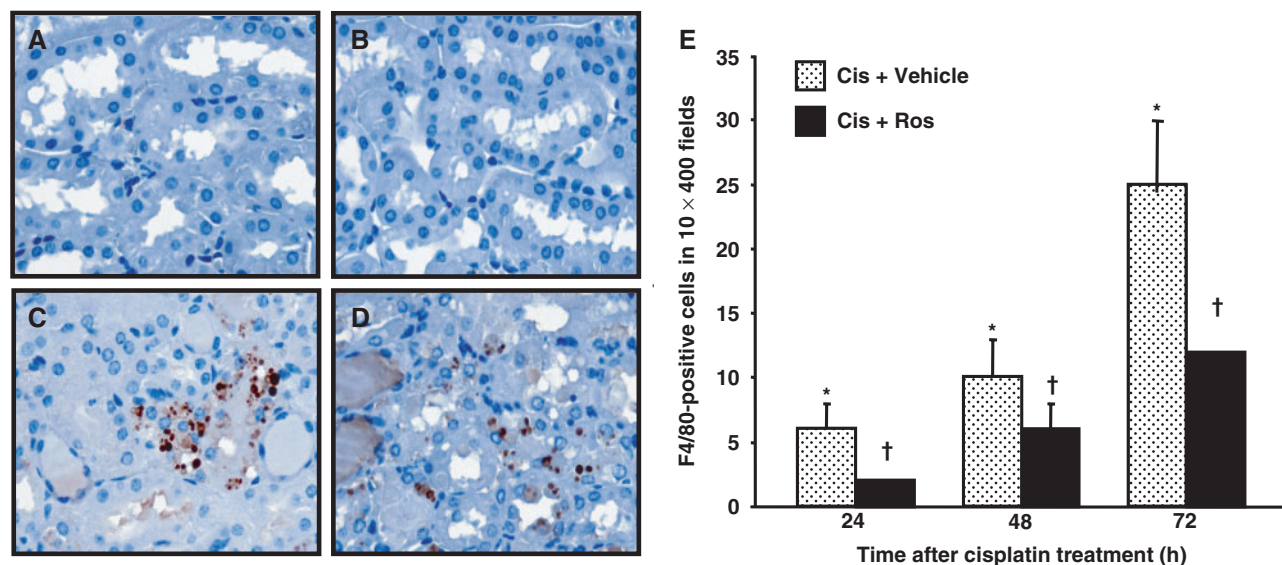


Fig. 5. Effect of rosiglitazone on infiltration of monocyte/macrophages in cisplatin-induced renal injury in mice. Mice were treated with (A) saline (Con), (B) rosiglitazone (Ros), (C) cisplatin or (D) cisplatin with rosiglitazone pre-treatment and sacrificed at 72 h. Note that the number of F4/80-stained cells is highest in panel C. Bars indicate 50 μ m. Magnification, 400 \times . (E) Number of F4/80-positive cells in 10 \times 400 fields at 24, 48 and 72 h after cisplatin administration. Data represent mean ± SEM from four independent experiments. ND, not detected. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin.

marked increase of ICAM-1 staining in proximal tubules, collecting ducts and interstitium (Figure 6D). Renal tissues obtained from mice administered cisplatin with rosiglitazone pre-treatment demonstrated

markedly reduced staining for ICAM-1 in interstitial and tubular epithelial cells when compared with renal tissues obtained from cisplatin-treated mice (Figure 6E).

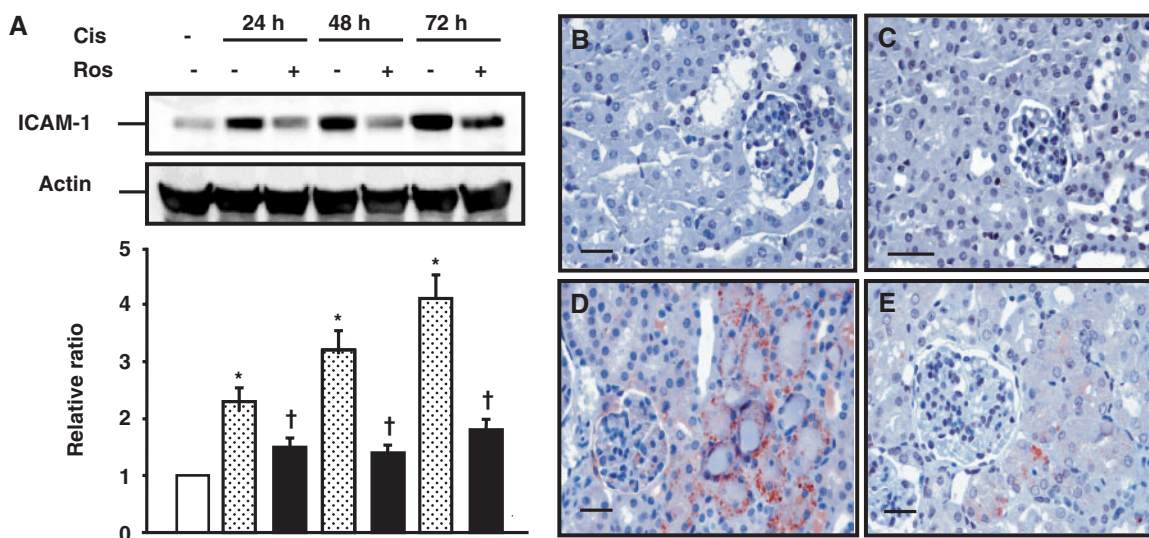


Fig. 6. Effect of rosiglitazone on expression of ICAM-1 in cisplatin-induced renal injury in mice. Western blot analysis of ICAM-1 in renal tissue at 24, 48 and 72 h after cisplatin administration (A). Renal tissue samples obtained from mice were homogenized, and total protein (50 μ g) was blotted and probed with anti-ICAM-1 antibody (upper panel). The blots were re-probed with an anti-actin antibody. Densitometric analyses are presented as the relative ratio of each protein to actin (lower panel). Data represent mean \pm SEM from four independent experiments. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin. (B–E) Immunohistochemical analysis of ICAM-1 in renal tissue at 72 h. Mice were treated with saline (B), rosiglitazone (C), cisplatin (D), or cisplatin with rosiglitazone pre-treatment (E). Note that the expression of ICAM-1 is highest in panel D. Pre-treatment with rosiglitazone significantly decreased the expression of ICAM-1 (E). Bars indicate 50 μ m. Magnification, 400 \times . Cis, cisplatin; Ros, rosiglitazone.

Effect of rosiglitazone on level of TNF- α in serum following cisplatin administration

A previous report has suggested that TNF- α mediates renal injury in cisplatin nephrotoxicity and that a blockade of TNF- α action ameliorates cisplatin nephrotoxicity [3]. We measured the level of TNF- α in serum at 24, 48 and 72 h after the administration of saline, rosiglitazone, cisplatin or cisplatin with rosiglitazone pre-treatment. Cisplatin injection increased TNF- α 3-fold in serum at 24 h compared with saline administration, and persisted until 72 h. However, pre-treatment with rosiglitazone significantly decreased serum levels of TNF- α in the cisplatin-treated group (Figure 7).

Effect of rosiglitazone on serum insulin and C-peptide level

Rosiglitazone is one of the anti-diabetic agents and reduces hyperglycaemia by decreasing insulin resistance in peripheral tissues. We investigated whether rosiglitazone affects serum insulin and C-peptide level in this study. Changes in serum insulin did not differ significantly in four experimental groups (mice treated with saline, 6.98 ± 0.88 μ IU/ml; rosiglitazone, 7.60 ± 1.68 μ IU/ml; cisplatin, 6.80 ± 1.11 μ IU/ml; cisplatin and rosiglitazone, 6.27 ± 1.30 μ IU/ml, $P > 0.05$). In addition, there were no significant differences in serum C-peptide level in four experimental groups (mice treated with saline, 1.52 ± 0.74 ng/ml; rosiglitazone, 1.25 ± 0.60 ng/ml; cisplatin, 1.38 ± 0.79 ng/ml;

cisplatin and rosiglitazone, 1.87 ± 0.80 ng/ml, $P > 0.05$).

Effect of rosiglitazone on NF- κ B activation in cisplatin nephrotoxicity

We assessed whether rosiglitazone inhibits the activation of the p65 subunit of NF- κ B. Our data showed that the phosphorylation of p65 increased 2.7-fold at 15 min of cisplatin treatment in HK-2 cells. Pre-treatment with rosiglitazone in cisplatin-treated HK-2 cells significantly decreased the phosphorylation of p65 compared with cisplatin-treated HK-2 cells alone (Figure 8A). Immunohistochemistry was carried out to confirm the result of the activated and translocated p65 in western blot. In cisplatin-treated mice, there were markedly increased positive stainings in nuclei of renal tubular cells. However, in rosiglitazone pre-treated mice group with cisplatin, nuclear stainings in renal tubular cells were significantly reduced compared with cisplatin-treated group (Figure 8B–E).

PPAR γ expression in kidney or HK-2 cells

We investigated whether PPAR γ , which is the binding target of rosiglitazone, is expressed in normal mouse kidney and HK-2 cells. As a positive control, PPAR γ protein was detected in the colon as evidenced by immunohistochemical and western blot analyses [15]. PPAR γ protein was also detected with similar

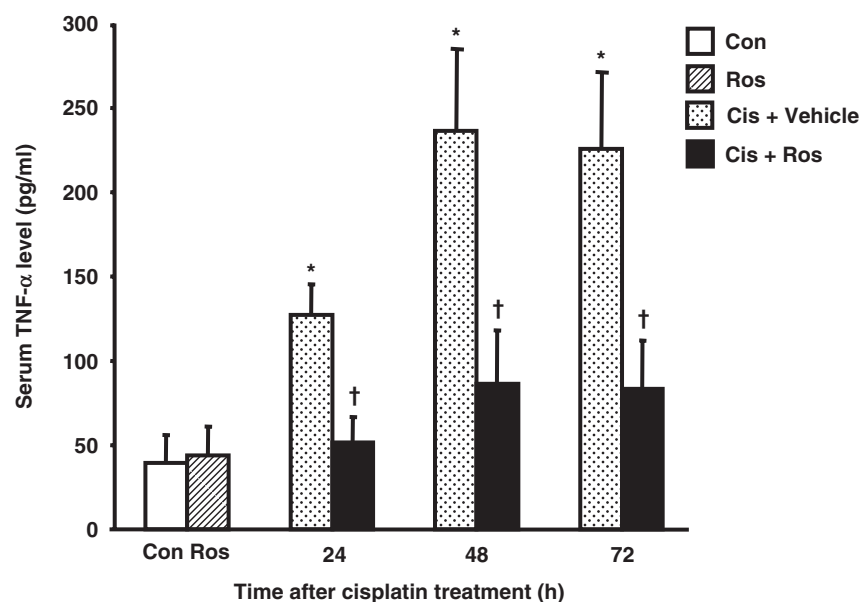


Fig. 7. Effect of rosiglitazone on the level of TNF- α in serum following cisplatin administration. TNF- α level in serum was measured at 24, 48 and 72 h after injection, $n = 5$ in each group. Data represent mean \pm SEM from four independent experiments. Con, saline; Ros, rosiglitazone; Cis, cisplatin; Cis \pm Ros, cisplatin injection with rosiglitazone. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin.

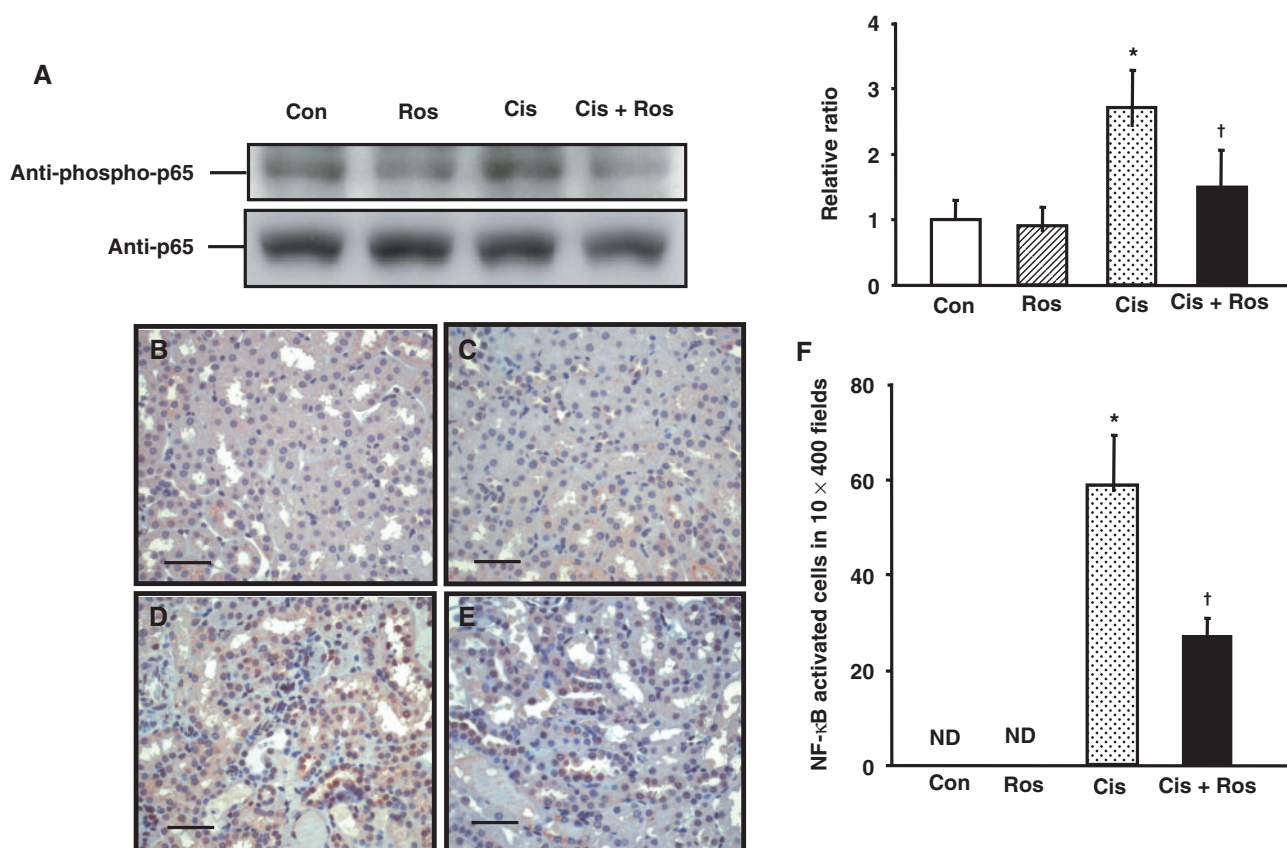


Fig. 8. Effect of rosiglitazone on cisplatin-induced NF- κ B activation in cisplatin nephrotoxicity. HK-2 cells, either untreated or pre-treated for 30 min with rosiglitazone (5 μ M) and then treated with cisplatin (1 μ g/ml) for 15 minutes, were used and analysed for p65 by western blot analysis (A; left panel). Densitometric analyses are presented (A; right panel). Con, saline; Ros, rosiglitazone; Cis, cisplatin; Cis \pm Ros, cisplatin injection with rosiglitazone pre-treatment. Values are expressed as mean \pm SEM. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin. Results were similar in four independent experiments. (B–E) Immunohistochemical detection of translocational NF- κ B p65 in renal tissues at 72 h after cisplatin administration. Bars indicate 50 μ m. Magnification, 400 \times . Mice were treated with saline (B), rosiglitazone (C), cisplatin (D), or cisplatin with rosiglitazone pre-treatment (E). In rosiglitazone pre-treated mice group with cisplatin, nuclear stainings in renal tubular cells were significantly reduced compared with cisplatin-treated group. (F) Number of NF- κ B activated cells in 10 \times 400 fields. Data represent mean \pm SEM from four independent experiments. ND, not detected. * $P < 0.05$ vs saline; † $P < 0.05$ vs cisplatin.

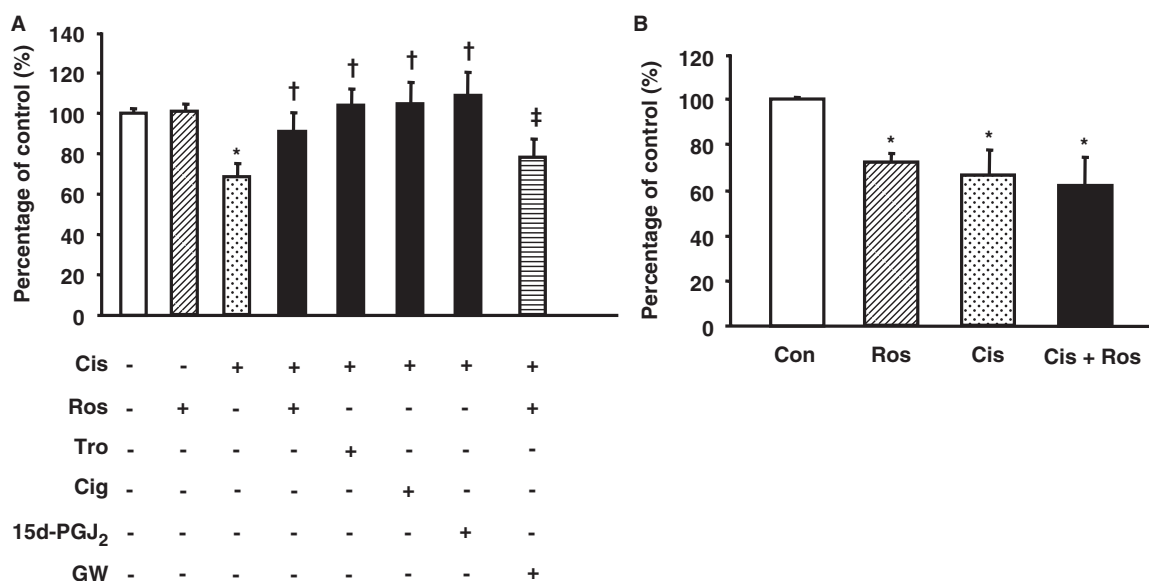


Fig. 9. Effect of PPAR γ agonists or PPAR γ antagonist on cell viability in cisplatin-treated HK-2 cells or 9L-glioma cells. Cell viability assay in cisplatin-treated HK-2 cells (A). The concentrations of rosiglitazone (Ros; 5 μ M), troglitazone (Tro; 5 μ M), ciglitazone (Cig; 5 μ M), 15-deoxy- Δ 12,14-prostaglandin J₂ (15d-PGJ₂; 5 μ M), or GW-9662 (GW; 2 μ M) were added to the cisplatin-treated HK-2 cells for 24 h. * P < 0.05 vs saline or rosiglitazone; † P < 0.05 vs cisplatin; ‡ P < 0.05 vs cisplatin \pm PPAR γ agonists. (B) Cell viability assay in 9L-glioma cells. * P < 0.05 vs saline.

abundance in epithelial cells of the kidney and HK-2 cells (data not shown).

Effect of PPAR γ agonists or PPAR γ antagonist on cell viability in cisplatin-treated HK-2 cells

We examined the effect of several PPAR γ agonists on cell survival in cisplatin-treated HK-2 cells using XTT assay. As shown in Figure 9A, rosiglitazone itself did not affect viability of HK-2 cells. Ciglitazone, troglitazone and 15d-PGJ₂ also had a protective effect in cisplatin-treated HK-2 cells similar to rosiglitazone. GW-9662, one of PPAR γ antagonists, blocked the viable effect of rosiglitazone in cisplatin-treated HK-2 cells. These findings suggest that PPAR γ agonists, including rosiglitazone, have a protective effect via a PPAR γ -dependent pathway in cisplatin-treated HK-2 cells. In addition, rosiglitazone did not reduce the anti-cancer effect of cisplatin in 9L-glioma cells (Figure 9B).

Discussion

In the present study, we have demonstrated that expression of ICAM-1 and TNF- α increase significantly after cisplatin treatment. Pre-treatment with rosiglitazone, a PPAR γ agonist, decreases the activation of NF- κ B in cisplatin-treated HK-2 cells and renal tissue, and also significantly reduces systemic over-production of TNF- α and renal expression of ICAM-1. In addition, rosiglitazone decreases the infiltration of monocyte/macrophages into the kidney of cisplatin-treated mice.

As cisplatin is an effective anti-neoplastic agent for the treatment of cancers, the major limitation of this drug is a dose-dependent and cumulative nephrotoxicity, sometimes requiring a reduction in dose or discontinuation of treatment.

It has been suggested that the pathogenesis of cisplatin nephrotoxicity has been associated with oxidative stress, DNA damage and apoptosis [16,17].

However, recent reports have shown that inflammation and expression of cytokines and chemokines have important roles in the pathogenesis of cisplatin nephrotoxicity. Ramesh and Reeves [3] have shown that cisplatin nephrotoxicity is characterized by the activation of pro-inflammatory cytokines and chemokines, and that TNF- α plays a central role in the activation of these cytokine responses. In addition, they have also revealed that salicylate acts via inhibition of TNF- α production to reduce cisplatin nephrotoxicity and the inhibition of TNF- α production may be mediated via stabilization of I κ B [5]. Jo *et al.* [18] have reported that the MAP-erk-kinase (MEK) inhibitor, U0126, has a significant functional and histological protection in cisplatin-induced renal injury by a decrease in TNF- α gene expression and inflammation. These findings strongly demonstrate the involvement of inflammatory reaction associated with pro-inflammatory cytokines, adhesion molecules or inflammatory cells in the development of cisplatin-induced renal injury. Thus, the agents that can down-regulate the activation of NF- κ B and TNF- α level are potential candidates for preventive or therapeutic intervention in cisplatin-induced renal injury.

Ligand-activated PPAR γ binds to a specific DNA binding site that leads to the regulation of the

transcription of numerous inflammatory genes such as cytokines and adhesion molecules. Recently, several studies have demonstrated that PPAR γ agonists exhibit an anti-inflammatory effect both *in vitro* and *in vivo* [7–9]. Our previous study has revealed that rosiglitazone reduces renal injury through anti-inflammatory mechanism in lipopolysaccharide-induced sepsis model [19].

It is known that TNF- α activates NF- κ B, and NF- κ B plays a critical role in inflammatory response. It is also known that activation of NF- κ B induces the expression of several genes, including TNF- α , ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1). Thus, the agents that can down-regulate TNF- α level and activation of NF- κ B are potential candidates for preventive or therapeutic intervention in cisplatin-induced renal injury. As expected, our results have shown that pre-treatment of rosiglitazone reduces cisplatin-induced expression of TNF- α and ICAM-1 and decreases the activation of NF- κ B. In this study, our data have shown that rosiglitazone decreases the activation of NF- κ B and reduces cisplatin-induced TNF- α levels, ICAM-1 expression and macrophage infiltration, which finally lead to functional and histological protection in cisplatin nephrotoxicity. In addition, studies have demonstrated that pathogenesis of cisplatin-induced renal injury is caused by apoptosis or genotoxic damage by either generation of reactive oxygen species or intercalation of DNA [2,16,20]. Although, the inflammatory responses induced by cisplatin may be involved in these processes, the exact causal mechanism of PPAR γ activation remains to be clarified. In our preliminary data, simultaneous administration of rosiglitazone in the cisplatin-treated group decreased significantly the BUN levels (cisplatin group, 182 ± 32 mg/dl vs cisplatin \pm rosiglitazone group, 62 ± 28 mg/dl), creatinine levels (cisplatin group, 2.8 ± 0.6 mg/dl vs cisplatin \pm rosiglitazone group, 1.3 ± 0.2 mg/dl) and histological damage (tubular necrosis score: cisplatin group, 2.7 ± 0.3 vs cisplatin \pm rosiglitazone group, 1.5 ± 0.2) compared with cisplatin alone. These results have shown that rosiglitazone may have therapeutic as well as preventive effects.

In conclusion, our study provides evidence that the inflammation by overproduction of TNF- α and activation of NF- κ B is one of the important determinants of cisplatin nephrotoxicity and that pre-treatment with rosiglitazone may be a therapeutic as well as a preventive strategy.

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Conflict of interest statement. None declared.

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