Transplant Immunology 54 (2019) 20–28

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

# Protective effect of rosiglitazone on chronic renal allograft dysfunction in rats

Jin Deng, Yue Xia, Qin Zhou, Xin Wang, Chongxiang Xiong, Xiaofei Shao, Mengjiao Shao, Hequn Zou*

*Department of Nephrology, The Third Affiliated Hospital of Southern Medical University, China*

ARTICLE INFO

*Keywords:*
Chronic renal allograft dysfunction
Interstitial fibrosis
Epithelial-to-mesenchymal transition
Inflammation
Rosiglitazone

ABSTRACT

*Background:* Chronic renal allograft dysfunction (CRAD) is the main condition affecting the long-term survival of renal allografts. Rosiglitazone, which is a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist, has been shown to exert antifibrotic and anti-inflammatory effects on some renal diseases. The present paper investigates the effect of rosiglitazone on CRAD using a murine model.

*Methods:* The CRAD group received classical orthotopic F344-Lewis kidney transplantation. The treatment group was treated with rosiglitazone for 12 weeks following renal transplantation. The control subjects were unine-phrectomized F344 and Lewis rats. Twelve weeks after the operation, the rats were harvested for renal function, histological, immunohistochemical and molecular biological analyses.

*Results:* Rosiglitazone treatment effectively decreased urine protein excretion and preserved renal function in the CRAD rats. Administration of rosiglitazone also inhibited interstitial fibrosis and macrophage infiltration in the CRAD rat kidneys. Furthermore, rosiglitazone treatment inhibited TGF-β and NF-κB pathway activation, decreased collagen I, collagen IV, α-SMA, MCP-1, ICAM-1, TNF-α, and IL-1β expression, and increased E-cadherin expression in renal allograft tissues from the CRAD rats.

*Conclusions:* Rosiglitazone successfully attenuates the development of CRAD via inhibition of TGF-β signaling, the renal tubular epithelial-to-mesenchymal transition (EMT), and inflammation.

## 1. Introduction

End-stage renal disease (ESRD) is a long-term, irreversible decline in kidney function and an important global health issue [1]. Kidney transplantation is the optimal therapeutic choice for patients with ESRD as a result of its considerable improvement of the quality of life and increased cost-effectiveness compared with those of dialysis [2,3]. Due to improvements in transplant techniques and the application of new, effective immunosuppressants, the 1-year survival rate of renal grafts has increased to 90%; however, the long-term survival rate has not significantly improved, and the 10-year survival rate remains < 65% [4]. Consequently, the long-term survival rate remains a challenge in clinical medicine. Chronic renal allograft dysfunction (CRAD) is the leading condition affecting the long-term survival of renal allografts [5]. The main clinical manifestations of CRAD are progressive deterioration of renal allograft function accompanied by proteinuria, hypertension, and progression to renal allograft failure that requires a second kidney transplant or dialysis. The main pathogenic features of CRAD are infiltration of mononuclear cells in the tubulointerstitium, interstitial fibrosis, tubular atrophy, glomerulosclerosis, mesangial matrix proliferation, and vascular intimal proliferation [6,7]. Currently, definitive and effective methods for control of CRAD progression are lacking. Thus, there remains a significant need to develop effective treatments to improve long-term outcomes following kidney transplantation.

Rosiglitazone, which is a commonly used antidiabetic agent, is an agonist of peroxisome proliferator-activated receptor-γ (PPAR-γ), which is widely expressed in renal tissues [8]. In addition to its glucose-lowering potency in diabetes, PPAR-γ activation also exerts antifibrotic and anti-inflammatory effects on nondiabetic renal diseases [9]. However, whether rosiglitazone mediates the development of CRAD is unknown. The main objective of the present study is to investigate the effects of rosiglitazone on the development of CRAD in rats.

* Corresponding author at: Department of Nephrology, The Third Affiliated Hospital of Southern Medical University, Guangzhou, Guangdong Province 510630, China.

*E-mail address:* hequnzou@hotmail.com (H. Zou).

https://doi.org/10.1016/j.trim.2019.01.002
Received 5 September 2018; Received in revised form 18 January 2019; Accepted 21 January 2019
Available online 22 January 2019

**Table 1**
Primers for selected genes.

| Gene | Forward primer | Reverse primer |
|---|---|---|
| TGF-β1 | 5′-CTGACCCCCACTGATACGC-3′ | 5′-CAGGTGTTGAGCCCTTTCC-3′ |
| MCP-1 | 5′-CTGACCCCAATAAGGAATG-3′ | 5′-TGAGGTGGTTGTGGAAAAGA-3′ |
| ICAM-1 | 5′-CAAACGGGAGATGAATGGT-3′ | 5′-TCTGGCGGTAATAGGTGTAAA-3′ |
| TNF-α | 5′-CTTCTCATTCCTGCTCGTGG-3′ | 5′-TCCGCTTGGTGGTTTGCTA-3′ |
| IL-1 | 5′-TTTGAGTCTGCACAGTTCCC-3′ | 5′-AACTATGTCCCGACCATTGC-3′ |
| GAPDH | 5′-CCTCGTCTCATAGACAAGATGGT-3′ | 5′-GGGTAGAGTCATACTGGAACATG-3′ |

## 2. Objective

Here, we aimed to investigate the effect of rosiglitazone on CRAD using a murine model.

## 3. Materials and methods

### 3.1. Animals

The animal experiments were approved by the Ethics Committee of the Third Affiliated Hospital of Southern Medical University. Naive male inbred rats weighing 150–220 g were purchased from the Experimental Animal Center of China (Peking, China). Fisher (F334) rats were used as donors, and Lewis (LEW) rats acted as graft recipients. The rats were housed under standard conditions with controlled light/dark cycles, temperature, and humidity and had unrestrained activity and free access to water and food.

### 3.2. Surgery

Orthotopic renal transplantation was performed using a professional-quality surgical microscope and a previously described method [7,10]. Briefly, rats were intraperitoneally anesthetized with a 3% pentobarbital-sodium injection (0.1 mL/100 g body weight). The left kidney of the donor F344 rat was isolated, removed, perfused with an ice-cold isotonic sodium chloride solution, and transplanted orthotopically into the host LEW rat, whose left renal vessels had been isolated and clamped and native kidney had been excised. Then, the donor and recipient renal artery, vein, and ureter were anastomosed end-to-end with 10–0 nonabsorbable prolene sutures. The transplanted kidney presented uniformly red after perfusion. No ureteral stent was used. The right kidney of the recipient was removed on day 10, at which time the transplanted kidney was assessed for surgical damage. Rats with any overt signs of an unsuccessful operation were excluded.

### 3.3. Experimental design

The animals were allocated into the following four experimental groups: (1) the F344 control group comprising uninephrectomized male F334 rats ($n = 5$), (2) the LEW control group comprising uninephrectomized male LEW rats (n = 5), (3) the CRAD group comprising male LEW rats that received an orthotopic left kidney transplant from male F334 donors ($n = 5$), and (4) the rosiglitazone group in which the animals were treated with rosiglitazone (5 mg/kg/d [11], GlaxoSmithKline Pharma AG, London, UK) after renal transplantation ($n = 5$). All rats were administered low doses (1.5 mg/kg/day) of cyclosporine A (Novartis Pharma AG, Basel, Switzerland) for the first 10 days and ceftriaxone sodium (1.5 mg/kg/d, Roche Pharma AG, Basel, Switzerland) for the first 3 days after renal transplantation to suppress acute rejection and infection, respectively. Animals were harvested 12 weeks after the operation, and then the transplanted and intact kidneys from the F344 and LEW control groups were resected.

### 3.4. Functional parameters

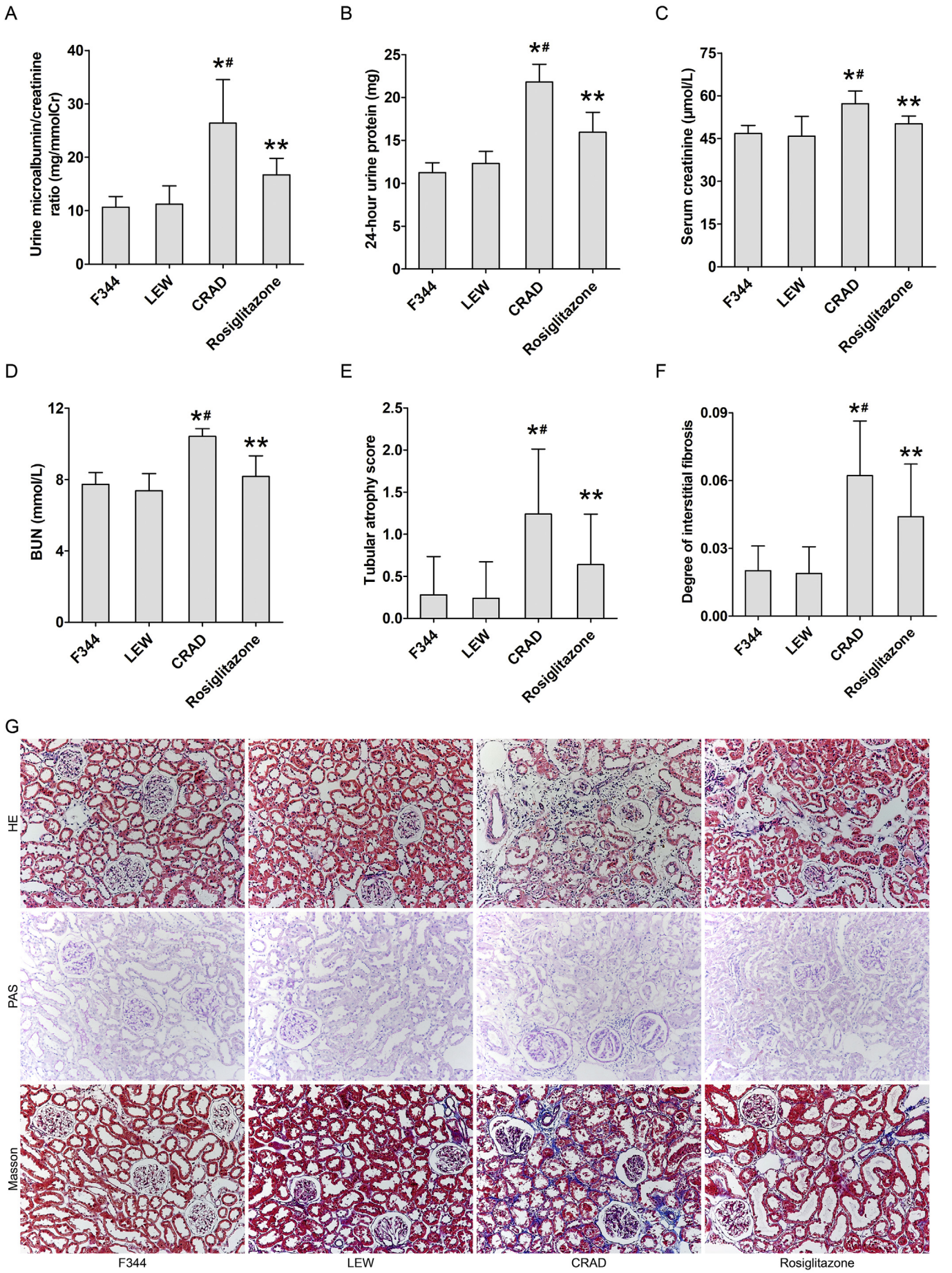
At week 12, the rats were placed in metabolic cages, and 24-h urine samples were collected. Then, the 24-h urinary protein output, urine microalbumin/creatinine ratio and serum creatinine and serum urea nitrogen levels were measured.

### 3.5. Histology and immunohistochemistry

Light microscopy was performed on 3-μm paraffin sections stained with hematoxylin–eosin (HE), periodic acid-Schiff (PAS), and Masson's trichrome. Renal interstitial fibrosis areas were quantified using Image-Pro Plus software (Media-Cybernetics, Silver Spring, MD, USA). Renal tubular atrophy was scored semiquantitatively on a scale from 0 to 3 according to the Banff 2015 criteria [12]. Ten randomly discontinuous fields per section of each renal tissue sample were evaluated. Three-micrometer sections of paraffin-embedded kidney tissues were transferred to a 10-mmol/L citrate buffer solution adjusted to a pH of 6.0. Then, the sections were microwaved for 20 min to retrieve antigens. A 3% hydrogen peroxide solution in methanol was applied for 20 min to block endogenous peroxidase activity. Thereafter, the kidney sections were labeled with anti-collagen IV (1:500, ab19808, Abcam, Cambridge, MA, USA), anti-TGF-β1 (1:15, sc-52,893, Santa Cruz Biotechnology, Dallas, TX, USA), anti-E-cadherin (1:200, 20,874-1-AP, Proteintech, Chicago, IL, USA), α-smooth muscle actin (α-SMA) (1:100, AF1032, Affinity Biosciences, Cincinnati, OH, USA), anti-p-NF-κB (p65) (1:500, ab86299, Abcam), or anti-CD68 (1:200, ab125212, Abcam) antibodies overnight at 4 °C. After washing with PBS, the secondary antibody was added to the sections, which were incubated for 30 min at 37 °C; then, the sections were washed with PBS. The slides were stained with 3,3′-diaminobenzidine (DAB, Golden Bridge Biotechnology Co., Beijing, China), counterstained with hematoxylin, dehydrated, cleared, and mounted. Positive areas were visualized by yellow staining.

### 3.6. Western blotting analysis

Total proteins were extracted from renal tissues using a commercial extraction kit. The protein concentrations of the samples were measured using the Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China). Equivalent amounts of protein were electrophoresed and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA). After blocking with 5% BSA, the membranes were probed with the following primary antibodies overnight at 4 °C: anti-collagen I (1:500, AF7001, Affinity Biosciences), anti-collagen IV (1:1000, ab19808, Abcam), anti-TGF-β1 (1:1000, sc-52,893, Santa Cruz Biotechnology), anti-smad3 (1:1000, R1510–24, Hangzhou HuaAn Biotechnology Co., Ltd., Hangzhou, China), anti-p-smad3 (1:1000, RT1568, Hangzhou HuaAn Biotechnology Co., Ltd.), anti-E-cadherin (1:1000, 20,874-1-AP, Proteintech), anti-α-SMA (1:1000, AF1032, Affinity Biosciences), anti-NF-κB (p65) (1:1000, ab16502, Abcam), anti-p-NF-κB (1:1000, ab86299, Abcam), anti-MCP-1 (1:2000, ab25124, Abcam), anti-ICAM-1 (1:2000, 10,020–1-AP, Proteintech), anti-TNF-α (1:1000, 60,291-1-Ig, Proteintech), anti-IL-1β (1:500, A13268, ABclonal Biotechnology, Woburn, MA, USA), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:5000, CWBIO,




*(caption on next page)*

**Fig. 1.** Rosiglitazone attenuates the progression of proteinuria, improves renal function, and ameliorates renal histological damage in CRAD rats. After 12 weeks, (A) the urine microalbumin/creatinine ratio and (B) the 24-h urinary protein, (C) serum creatinine, and (D) blood urea nitrogen levels were measured. (E) The percentages of interstitial fibrosis and (F) tubular atrophy score in the different groups. (G) Photomicrographs illustrating hematoxylin–eosin (HE), periodic acid-Schiff (PAS), and Masson's trichrome staining of kidney tissue from the different groups (original magnification ×200).Data are represented as means ± standard deviations (SDs). *$p < .05$ versus F344 control group; #p < .05 versus LEW control group. **p < .05 versus CRAD group.

Beijing, China), mouse monoclonal anti-β-actin (1:5000, CWBIO), or mouse monoclonal anti-β-tubulin (1:5000, Proteintech). After washing, the membrane was subsequently incubated with corresponding secondary antibody (1:5000, CWBIO) for 60 min at room temperature. The immunoreactive bands were visualized using enhanced chemiluminescence signals captured using a digital visualizer (Eastman Kodak Company, USA) and quantified using the ImageJ software version 1.46 (Wayne Rasband, National Institutes of Health, USA). All experiments were repeated three times.

### 3.7. RT-PCR

Total RNA was extracted from whole kidney tissue samples with TRIzol (TaKaRa, Dalian, China), and mRNAs were reversed transcribed into cDNAs using the Transcriptor First Strand cDNA synthesis kit (TaKaRa). The nucleotide sequences of all primers used in this study are shown in Table 1.

### 3.8. Statistical analysis

All data are presented as means ± standard deviations (SDs) and were analyzed using the SPSS 20 software. Comparisons among groups were performed using one-way ANOVA. $p < .05$ was considered significant.

## 4. Results

### 4.1. Rosiglitazone prevents renal dysfunction and proteinuria in CRAD rats

The 24-h urinary protein output, urine microalbumin/creatinine ratio and serum creatinine and serum urea nitrogen levels were significantly increased in the CRAD group compared with the control group levels (Fig. 1A-D). The results were consistent with those of previous studies [6,7]. As shown in Fig. 1A-D, administration of rosiglitazone significantly reduced the 24-h urinary protein output, urine microalbumin/creatinine ratio and serum creatinine and serum urea nitrogen levels in the CRAD rats. Taken together, our data indicate that rosiglitazone can improve renal function and reduce proteinuria in rats with CRAD.

### 4.2. Rosiglitazone ameliorates renal histological damage in CRAD rats

As shown in Fig. 1E-G. No marked histological changes were observed in the kidney sections from the F344 and LEW control rats. The main histological disorders in the kidney sections from the CRAD rats were limited interstitial fibrosis, tubular atrophy and mononuclear cell infiltration. These lesions and tissue damage were strikingly reduced in the rosiglitazone group. These pathological findings in the kidney suggest that rosiglitazone exerts a protective effect in CRAD rats and prevents renal histological damage.

### 4.3. Rosiglitazone attenuates the progression of renal fibrosis in CRAD rats

Renal interstitial fibrosis, which is characterized by excessive ECM deposition, is the common pathological feature of all types of CKD, including CRAD [13,14]. To examine whether rosiglitazone alleviated renal fibrogenesis in CRAD rats, ECM protein deposition and expression were examined in the kidneys of the CRAD rats. As shown in Fig. 1F-G, Masson staining showed that renal interstitial fibrosis was significantly more obvious in the CRAD group than in the control group. And administration of rosiglitazone significantly improved the morphologic lesions, with less fibrosis observed in the interstitium. We further examined collagen I and collagen IV expression by western blotting and immunohistochemistry. The results indicated that collagen I and collagen IV expression was increased in the kidney of the CRAD rats, and administration of rosiglitazone significantly reduced their expression (Fig. 2A-C). Therefore, our data show that rosiglitazone attenuates the progression of renal fibrosis in CRAD rats.

### 4.4. Rosiglitazone abrogates the TGF-β/Smad3 signaling pathway in the kidneys of CRAD rats

It is well documented that TGF-β signaling plays a critical role in renal fibrosis [15], but whether rosiglitazone inhibits TGF-β signaling in CRAD remains unclear. We first investigated the effect of rosiglitazone on TGF-β expression in the kidney of CRAD rats using western blotting, immunohistochemistry, and RT-PCR. As shown in Fig. 2C-E, TGF-β1 expression was increased in the kidney of CRAD rats and suppressed by rosiglitazone treatment. Because Smad3 is the major downstream mediator of TGF-β signaling and directly binds to specific sites in promoter regions to regulate transcription of genes involved in the fibrotic response, such as collagen I [16]. We next analyzed the level of phosphorylated Smad3 (p-Smad3) in the kidney of each group of rats. As shown in Fig. 2F, the level of p-Smad3 in the kidney of CRAD rat was up-regulated and rosiglitazone administration significantly reduced its expression. Therefore, rosiglitazone can inhibit TGF-β/Smad3 signaling in CRAD.

### 4.5. Rosiglitazone inhibits the renal tubular epithelial-to-mesenchymal transition in the kidney of CRAD rats

The renal tubular epithelial-to-mesenchymal transition (EMT) is defined as the process during which renal tubular epithelial cells lose their epithelial phenotype and the expression of junction markers, such as E-cadherin, and acquire mesenchymal cell characteristics, such as an increase in expression of α-SMA, which leads to secretion of ECM proteins [17]. Increasing evidence has shown that the renal tubular EMT plays a very important role in the pathogenesis of renal interstitial fibrosis in CRAD [18,19]. To examine whether rosiglitazone inhibited the renal tubular EMT, we investigated E-cadherin and α-SMA expression in the kidney of each group of rats by western blotting and immunohistochemistry. The results showed decreased E-cadherin expression in the CRAD rats, whereas rosiglitazone treatment increased E-cadherin expression (Fig. 3A and B). In contrast, α-SMA expression was significantly higher in the CRAD group than in the control group, and rosiglitazone treatment reduced α-SMA expression (Fig. 3B and C). Based on these findings, our data indicate that rosiglitazone inhibits the renal tubular EMT in the kidney of CRAD rats.

### 4.6. Rosiglitazone mediates NF-κB pathway activation and inhibits macrophage infiltration in the kidney of CRAD rats

NF-κB is a key transcription factor in inflammatory response, and its activation increases leukocyte infiltration, inflammation, and cytokine, chemokine, and adhesion molecule gene expression [20]. Expression of phosphorylated NF-κB (p-NF-κB p65) was increased in the kidney of the CRAD rats, and rosiglitazone significantly reduced its expression (Fig. 4A). The total NF-κB (p65) expression level was not changed in the

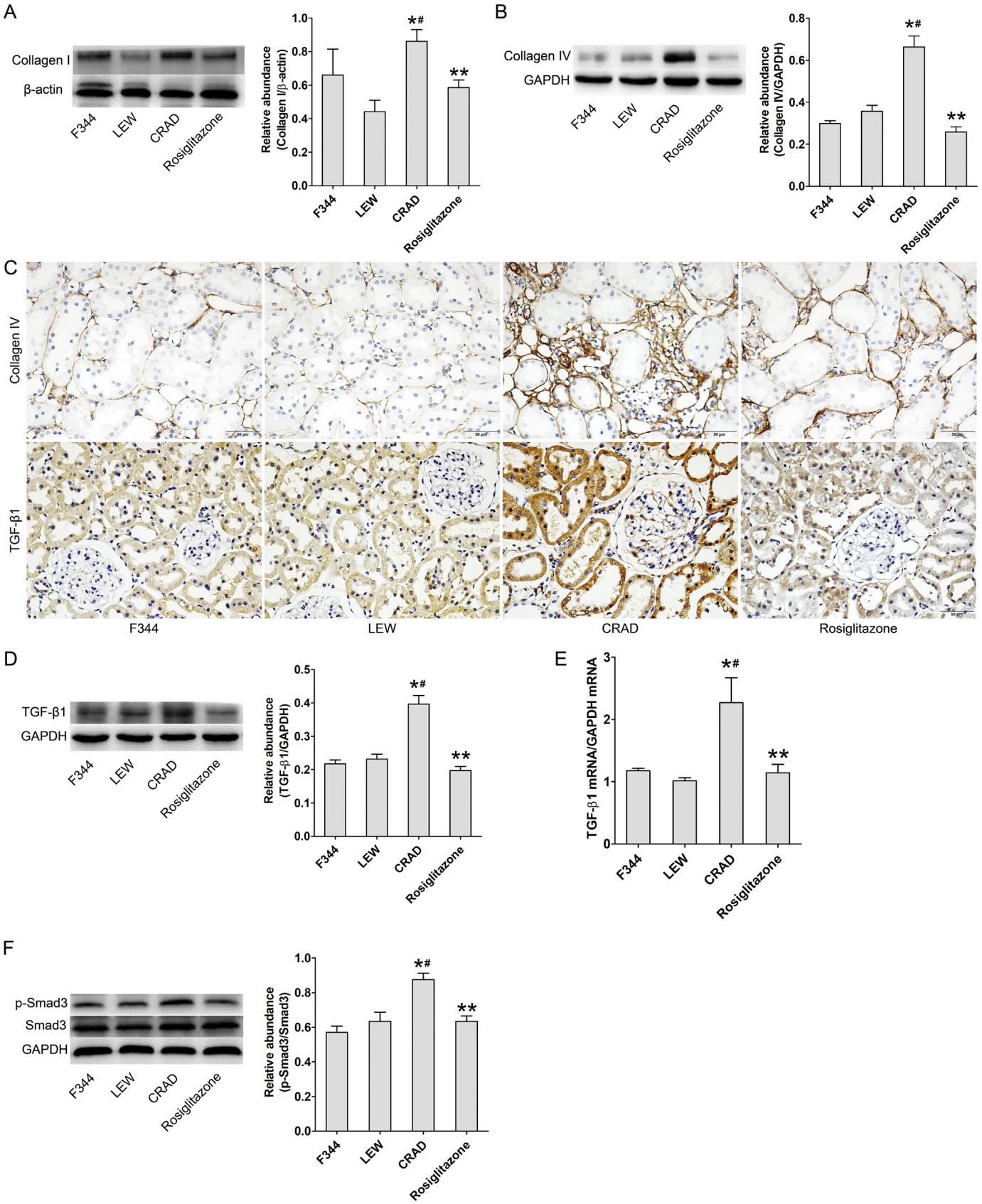



**Fig. 2.** Rosiglitazone decreases collagen I and collagen IV expression, and inhibits the TGF-β/Smad3 signaling pathway in the kidney of CRAD rats. The kidneys were collected for immunoblotting analysis of (A) collagen I, (B) collagen IV, (D) TGF-β1, (F) Smad3, and p-Smad3. (C) Photomicrographs illustrating collagen IV and TGF-β1immunochemistry staining of kidney tissue from the different groups (original magnification ×400). (E) TGF-β1 mRNA expression in renal tissue from the different groups (normalized to GAPDH). Data are represented as means ± standard deviations (SDs). *p < .05 versus F344 control group; #p < .05 versus LEW control group. **p < .05 versus CRAD group.

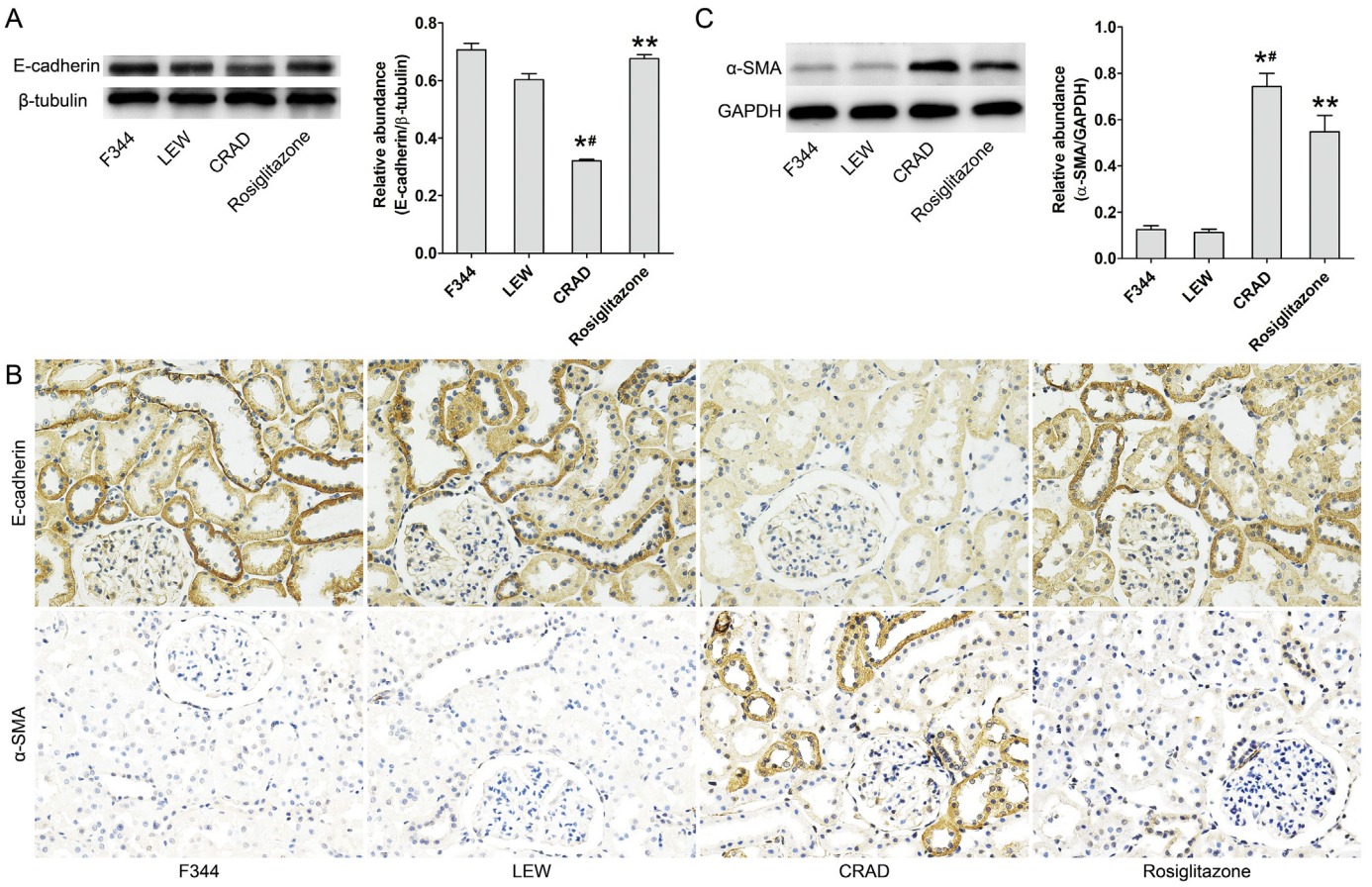



**Fig. 3.** Rosiglitazone inhibits the renal tubular epithelial-to-mesenchymal transition (EMT) in the kidney of CRAD rats. The kidneys were collected for immunoblotting analysis of (A) E-cadherin and (C) α-SMA. (B) Photomicrographs illustrating E-cadherin and α-SMA immunochemistry staining of kidney tissue from the different groups (original magnification ×400). Data are represented as means ± standard deviations (SDs). *p < .05 versus F344 control group; #p < .05 versus LEW control group. **p < .05 versus CRAD group.

kidney from each group of rats (Fig. 4A). Furthermore, the CRAD rats exhibited a marked increase in positive p-NF-κB (p65) staining in the nuclei of renal tubular cells compared with the cells from the control rats. Nuclear staining of renal tubular cells was significantly reduced in the rosiglitazone-treated group compared with that in the CRAD group (Fig. 4B).

Inflammatory cell infiltration in the renal interstitium is a common pathologic feature of almost all kinds of CKD, including CRAD [21,22]. Increasing evidence shows that infiltration of mononuclear cells, particularly macrophages, contributes to the development of interstitial fibrosis and poor outcomes of renal transplantation in humans and animal models [23–27]. To assess whether rosiglitazone inhibited macrophage infiltration in CRAD, CD68, which is a marker of active macrophages, was examined in the kidney of each group of rats using immunohistochemistry. An increased number of macrophages was found in the kidney of the CRAD rats, and rosiglitazone treatment significantly inhibited their infiltration (Fig. 4B).

Taken together, our data illustrate that rosiglitazone can mediate the NF-κB signaling pathway and inhibit macrophage infiltration in the renal interstitium in rats with CRAD.

### *4.7. Rosiglitazone inhibits the release of cytokines/chemokines in the kidney of CRAD rats*

It has been proved that proinflammatory cytokines/chemokines are essential for the development of CRAD [28–31]. Thus, we examined the expression of some proinflammatory cytokines/chemokines, including MCP-1, ICAM-1, TNF-α, and IL-1β, in the kidney by western blotting and RT-PCR. The results showed that these levels were significantly increased in the CRAD group compared with the F344 and LEW control group levels. Administration of rosiglitazone to the CRAD rats resulted in decreased MCP-1, ICAM-1, TNF-α, and IL-1β levels (Figs. 4C-J). Based on these findings, rosiglitazone suppresses the expression of proinflammatory cytokines/chemokines in CRAD rats.

## 5. Discussion

The long-term survival rates of renal grafts are still a challenge in clinical medicine. CRAD significantly affects long-term graft survival. In the present study, we investigated the effects of the PPAR-γ agonist rosiglitazone on a CRAD rat model. We established the CRAD model in Fisher 344 and Lewis rats. The urinary protein, serum creatinine, and serum urea nitrogen levels were elevated with the progression of CRAD at 12 weeks post-surgery. The pathological tests of the transplanted kidneys showed interstitial fibrosis and obvious mononuclear cell infiltration. Thus, we successfully established our CRAD rat model, and our results were consistent with those of previous studies [6,7,28]. Based on the results of the current study, rosiglitazone treatment effectively decreased urine protein excretion and preserved renal function in the CRAD rats. And rosiglitazone significantly inhibited interstitial fibrosis and macrophage infiltration in the kidneys of the CRAD rats. Furthermore, rosiglitazone mediated TGF-β and NF-κB pathway activation, decreased collagen I, collagen IV, α-SMA, MCP-1, ICAM-1, TNF-α, and IL-1β expression, and increased E-cadherin expression in renal allograft tissues from the CRAD rats.

PPAR-γ is a member of the ligand-activated nuclear hormone

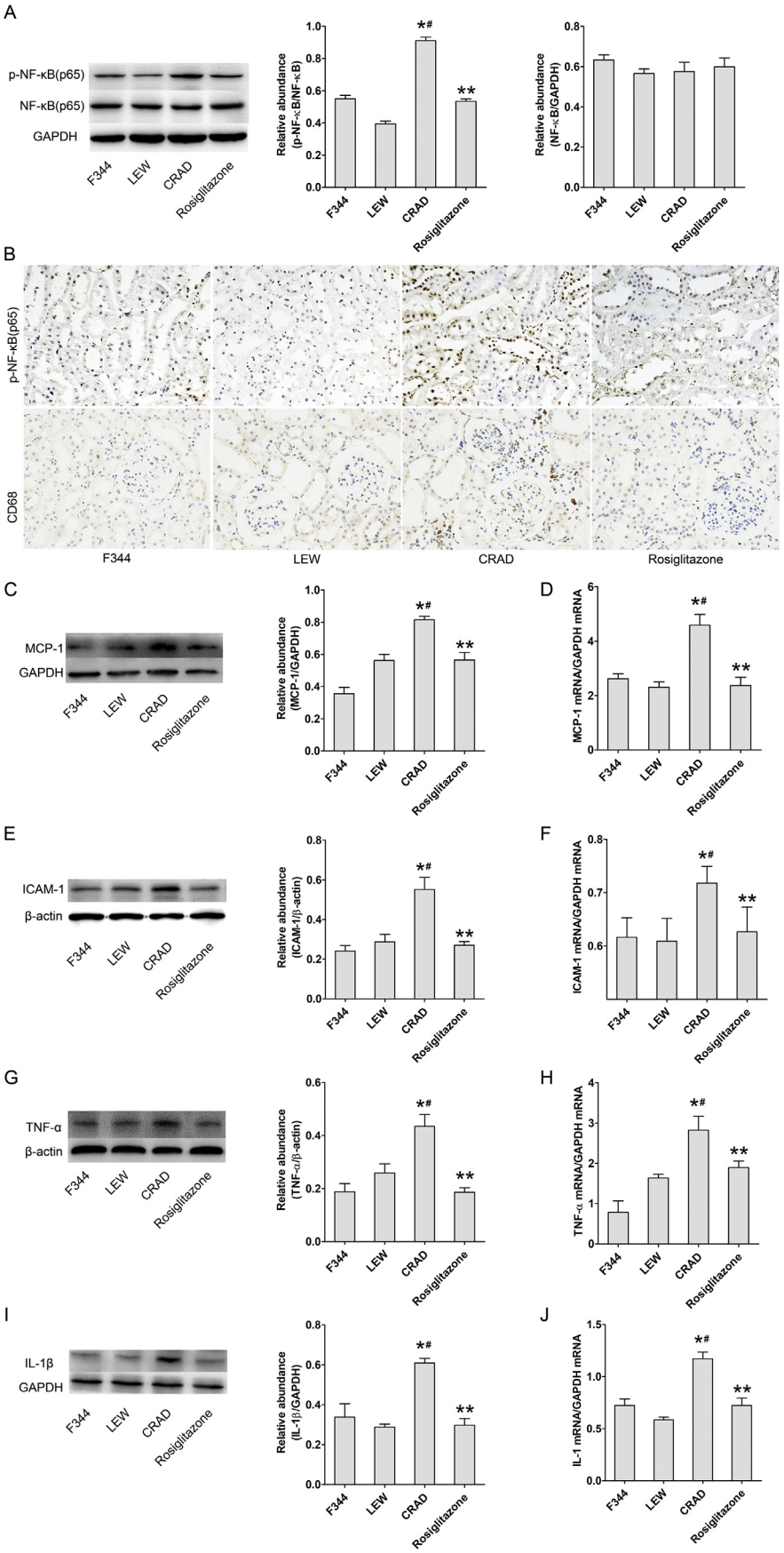



**Fig. 4.** Rosiglitazone inhibits NF-κB activation, macrophage infiltration, and the release of cytokines/chemokines in the kidney of CRAD rats. The kidneys were collected for immunoblotting analysis of (A) p-NF-κB (p65), NF-κB (p65), (C) MCP-1, (E) ICAM-1, (G) TNF-α, and (I) IL-1β. (B) Photomicrographs illustrating p-NF-κB (p65) and CD68 immunochemistry staining of kidney tissue from the different groups (original magnification ×400). (D) MCP-1, (F) ICAM-1, (H) TNF-α, and (J) IL-1 mRNA expression in renal tissue from the different groups (normalized to GAPDH). Data are represented as means ± standard deviations (SDs). *p < .05 versus F344 control group; #p < .05 versus LEW control group. **p < .05 versus CRAD group.

receptors. Ligand-activated PPAR-γ binds to a specific DNA binding site, termed the peroxisome proliferator response element (PPRE), to regulate the transcription of numerous target genes that involve many biological effects, such as inflammation, fibrosis, and proliferation. PPAR-γ agonists have been reported to exert antifibrotic effects on nondiabetic renal diseases by reducing TGF-β1 and collagen IV secretion [32–34]. As shown in the study by Zafiriou S et al., a PPAR-γ agonist inhibited cell growth and reduced matrix production in human kidney fibroblasts [35]. Additionally, rosiglitazone has been reported to have a protective effect against cyclosporine-induced pancreatic and renal injury by decreasing TGF-β1 expression [36]. Moreover, in the study by Lee et al., rosiglitazone, a PPAR-γ agonist, reduced renal injury and macrophage/monocyte infiltration in the kidney in a lipopolysaccharide-induced mouse sepsis model by inhibiting NF-κB activation [37]. Rosiglitazone also decreased NF-κB activation and reduced cisplatin-induced increases in the TNF-α levels, ICAM-1 expression, and macrophage infiltration, which ultimately led to functional and histological protection from cisplatin nephrotoxicity [38]. However, as a PPAR-γ agonist, the effect of rosiglitazone in the progression of CRAD is still unclear.

Interstitial fibrosis is the common pathological change of all types of renal diseases, including CRAD. TGF-β is an important factor that is required for the development of glomerulosclerosis and interstitial fibrosis in various kidney diseases. By measuring the TGF-β mRNA levels in renal biopsy specimens at different time points after renal transplantation, Baboolal et al. found that the TGF-β mRNA levels were significantly increased during the early stage of allograft injury [39]. As shown in the study by Mas et al., the pathological interstitial fibrosis and tubular atrophy (IFTA) score and urine protein excretion were correlated with TGF-β1 expression in allografts [40]. Our previous study also suggested that TGF-β1 played an important role in the interstitial fibrosis and tubular atrophy of renal allografts beginning in the early stage of CRAD [41]. On the other hand, ECM overaccumulation plays an important role in the development of IFTA and leads to fibrosis of renal allografts and gradual allograft loss. Collagen I and collagen IV are the major collagen component in renal tissues. As shown in our previous study of CRAD patients, collagen IV deposition in the renal interstitium may be an important step in the renal allograft fibrosis process [41]. In the present study, we found diffuse interstitial fibrosis, enhanced TGF-β activation, and increased expression of collagen I and collagen IV in renal allografts. Furthermore, rosiglitazone treatment significantly alleviated interstitial fibrosis, inhibited TGF-β activation, and decreased expression of collagen I and collagen IV in renal allograft tissues from CRAD rats.

There is growing body of evidence that the renal tubular EMT is an important source of fibrogenesis and an important event in the pathogenesis of renal interstitial fibrosis in CRAD patients [19,42]. Our data demonstrated that a significant decrease in E-cadherin expression and increase in α-SMA expression occurred in the kidneys of CRAD rats. These findings suggest that the renal tubular EMT occurs in the kidneys of CRAD rats and that the EMT may play an important role in CRAD progression. Furthermore, rosiglitazone treatment significantly increased E-cadherin expression and decreased α-SMA expression. Our study demonstrated for the first time that rosiglitazone inhibited the renal tubular EMT in CRAD. That may constitute the mechanism by which rosiglitazone attenuates renal fibrosis of CRAD.

Based on convincing evidence, inflammation is not only a key and final common pathway driving progressive nephron loss but is also an important predictor of subsequent allograft fibrosis in a renal allograft [43–46]. NF-κB is a key transcription factor that initiates the inflammatory response by inducing the expression of a variety of proinflammatory genes and mediators, including chemokines, proinflammatory cytokines, and adhesion molecules [47]. NF-κB activation is an important cell-signaling event during the proinflammatory response to CRAD and is closely related to the development and progression of CRAD [48,49]. NF-κB deficiency significantly attenuates the inflammatory response, alleviates tissue damage, and improves the survival of allografts, including kidney allografts [50]. Recently, MCP-1 was shown to be involved in CRAD pathogenesis, and its high expression was closely related to the inflammatory cell infiltration, interstitial fibrosis, and tubular atrophy, which were induced by CRAD [29]. In addition, IL-1 is a proinflammatory cytokine that is involved in various renal diseases, including CRAD, through recruitment of inflammatory factors [31,51]. IL-1β and TNF-α mRNA expression in the arterial wall of the renal arteries is upregulated in CRAD patients [52]. In the present study, obvious mononuclear cell infiltration, enhanced NF-κB activation, and increased MCP-1, ICAM-1, TNF-α, and IL-1β expression were observed in renal allografts. Administration of rosiglitazone inhibited NF-κB activation and decreased MCP-1, ICAM-1, TNF-α, and IL-1β expression in renal allograft tissues from CRAD rats. These data suggest that inhibition of the inflammatory response may serve as a mechanism by which rosiglitazone attenuates the pathogenesis and renal fibrosis of CRAD.

In conclusion, we have demonstrated that rosiglitazone attenuates the development of CRAD in a rat model. This effect was associated with inhibition of TGF-β signaling, the renal tubular EMT, and inflammation. Thus, rosiglitazone may represent a novel therapeutic strategy for the early prevention and treatment of CRAD.

## Conflict of interest

All the authors declared no competing interests.

## Acknowledgments

This study was supported by the following Science Foundations: 1. Provincial Major Projects of Education Department of Guangdong Province (Natural Science), 2014KZDXM019, 2015; 2. Science and Technique Program of Guangzhou, 201604020015, 2015; 3. Shenzhen 2013 Oversees High Level Talents “Peacock” Project, 21030409 1733248164; 4. The Natural Science Foundation of Guangdong Province, 2016A030313559, 2016; 5. South Wisdom Valley Innovative Research Team Program, CXTD-004, 2014; 6. Risk factors and prediction model of chronic kidney disease caused by metabolic syndrome: A multicentric prospective cohort study Clinical trial training project of Southern Medical University, LC2016PY047, 2016.

## References

[1] A. Levin, M. Tonelli, J. Bonventre, J. Coresh, J.A. Donner, A.B. Fogo, C.S. Fox, R.T. Gansevoort, H.J.L. Heerspink, M. Jardine, B. Kasiske, A. Kottgen, M. Kretzler, A.S. Levey, V.A. Luyckx, R. Mehta, O. Moe, G. Obrador, N. Pannu, C.R. Parikh, V. Perkovic, C. Pollock, P. Stenvinkel, K.R. Tuttle, D.C. Wheeler, K.U. Eckardt, Global kidney health 2017 and beyond: a roadmap for closing gaps in care, research, and policy, Lancet 390 (10105) (2017) 1888–1917.

[2] R.A. Wolfe, V.B. Ashby, E.L. Milford, A.O. Ojo, R.E. Ettenger, L.Y. Agodoa, P.J. Held, F.K. Port, Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant, N. Engl. J. Med. 341 (23) (1999) 1725–1730.

[3] B.L. Kasiske, M.G. Zeier, J.R. Chapman, J.C. Craig, H. Ekberg, C.A. Garvey, M.D. Green, V. Jha, M.A. Josephson, B.A. Kiberd, H.A. Kreis, R.A. McDonald, J.M. Newmann, G.T. Obrador, F.G. Vincenti, M. Cheung, A. Earley, G. Raman, S. Abariga, M. Wagner, E.M. Balk, KDIGO clinical practice guideline for the care of kidney transplant recipients: a summary, Kidney Int. 77 (4) (2010) 299–311.

[4] A. Hart, J.M. Smith, M.A. Skeans, S.K. Gustafson, D.E. Stewart, W.S. Cherikh, J.L. Wainright, A. Kucheryavaya, M. Woodbury, J.J. Snyder, B.L. Kasiske, A.K. Israni, OPTN/SRTR 2015 Annual Data Report: Kidney, Am. J. Transplant. 17 (Suppl. 1) (2017) 21–116.

[5] B.J. Nankivell, D.R.J. Kuypers, Diagnosis and prevention of chronic kidney allograft loss, Lancet 378 (9800) (2011) 1428–1437.

[6] D. Gu, Y. Shi, Y. Ding, X. Liu, H. Zou, Dramatic early event in chronic allograft nephropathy: increased but not decreased expression of MMP-9 gene, Diagn. Pathol. 8 (1) (2013) 13.

[7] L.N. Zhou, N. Wang, Y. Dong, Y. Zhang, H. Zou, Q. Li, Y. Shi, L. Chen, W. Zhou, C. Han, Increased Expression of p-Akt correlates with Chronic Allograft Nephropathy in a Rat Kidney Model, Cell Biochem. Biophys. 71 (3) (2015) 1685–1693.

[8] W.A. Hsueh, S.B. Nicholas, Peroxisome proliferator-activated receptor-gamma in

the renal mesangium, Curr. Opin. Nephrol. Hypertens. 11 (2) (2002) 191–195.
[9] P.A. Sarafidis, G.L. Bakris, Protection of the kidney by thiazolidinediones: an assessment from bench to bedside, Kidney Int. 70 (7) (2006) 1223–1233.
[10] Y. Xia, J. Deng, Q. Zhou, X. Shao, X. Yang, M. Sha, H. Zou, Expression and significance of Sirt1 in renal allografts at the early stage of chronic renal allograft dysfunction, Transpl. Immunol. 48 (2018) 18–25.
[11] S. Efrati, S. Berman, A. Chachashvili, N. Cohen, Z. Averbukh, J. Weissgarten, Rosiglitazone Treatment Attenuates Expression of Inflammatory Hallmarks in the remaining Kidney following Contralateral Nephrectomy, Am. J. Nephrol. 28 (2) (2008) 238–245.
[12] A. Loupy, M. Haas, K. Solez, L. Racusen, D. Glotz, D. Seron, B.J. Nankivell, R.B. Colvin, M. Afrouzian, E. Akalin, N. Alachkar, S. Bagnasco, J.U. Becker, L. Cornell, C. Drachenberg, D. Dragun, H. de Kort, The Banff 2015 Kidney meeting Report: current challenges in rejection Classification and prospects for adopting Molecular Pathology, Am. J. Transplant. 17 (1) (2017) 28–41.
[13] A.A. Eddy, Overview of the cellular and molecular basis of kidney fibrosis, Kidney Int. Suppl. 4 (1) (2014) 2–8.
[14] Y.Y. Wang, H. Jiang, J. Pan, X.R. Huang, Y.C. Wang, H.F. Huang, K.F. To, D.J. Nikolic-Paterson, H.Y. Lan, J.H. Chen, Macrophage-to-Myofibroblast transition contributes to interstitial fibrosis in chronic renal allograft injury, J. Am. Soc. Nephrol. 28 (7) (2017) 2053–2067.
[15] X.M. Meng, P.M. Tang, J. Li, H.Y. Lan, TGF-beta/Smad signaling in renal fibrosis, Front. Physiol. 6 (2015) 82.
[16] Y. Zhang, X.R. Huang, L.H. Wei, A.C. Chung, C.M. Yu, H.Y. Lan, miR-29b as a therapeutic agent for angiotensin II-induced cardiac fibrosis by targeting TGF-beta/Smad3 signaling, Mol. Ther. 22 (5) (2014) 974–985.
[17] R.C. Stone, I. Pastar, N. Ojeh, V. Chen, S. Liu, K.I. Garzon, M. Tomic-Canic, Epithelial-mesenchymal transition in tissue repair and fibrosis, Cell Tissue Res. 365 (3) (2016) 495–506.
[18] Z. Xu, C. Zhao, Z. Wang, J. Tao, Z. Han, W. Zhang, R. Tan, M. Gu, Interleukin-33 levels are elevated in chronic allograft dysfunction of kidney transplant recipients and promotes epithelial to mesenchymal transition of human kidney (HK − 2) cells, Gene 644 (2018) 113–121.
[19] C. Zhao, Z. Xu, Z. Wang, C. Suo, J. Tao, Z. Han, M. Gu, R. Tan, Role of tumor necrosis factor-alpha in epithelial-to-mesenchymal transition in transplanted kidney cells in recipients with chronic allograft dysfunction, Gene 642 (2018) 483–490.
[20] H. Huang, Y. Liu, J. Daniluk, S. Gaiser, J. Chu, H. Wang, Z.S. Li, C.D. Logsdon, B. Ji, Activation of nuclear factor-kappaB in acinar cells increases the severity of pancreatitis in mice, Gastroenterology 144 (1) (2013) 202–210.
[21] S.R. Mulay, A. Evan, H.J. Anders, Molecular mechanisms of crystal-related kidney inflammation and injury. Implications for cholesterol embolism, crystalline nephropathies and kidney stone disease, Nephrol. Dial. Transplant. 29 (3) (2014) 507–514.
[22] X.M. Meng, D.J. Nikolic-Paterson, H.Y. Lan, Inflammatory processes in renal fibrosis, Nat. Rev. Nephrol. 10 (9) (2014) 493–503.
[23] D. Toki, W. Zhang, K.L. Hor, D. Liuwantara, S.I. Alexander, Z. Yi, R. Sharma, J.R. Chapman, B.J. Nankivell, B. Murphy, P.J. O'Connell, The role of macrophages in the development of human renal allograft fibrosis in the first year after transplantation, Am. J. Transplant. 14 (9) (2014) 2126–2136.
[24] R. Girlanda, D.E. Kleiner, Z. Duan, E.A. Ford, E.C. Wright, R.B. Mannon, A.D. Kirk, Monocyte infiltration and kidney allograft dysfunction during acute rejection, Am. J. Transplant. 8 (3) (2008) 600–607.
[25] P.J. Matheson, I.D. Dittmer, B.W. Beaumont, M.J. Merrilees, H.L. Pilmore, The macrophage is the predominant inflammatory cell in renal allograft intimal arteritis, Transplantation 79 (12) (2005) 1658–1662.
[26] E. Guillen-Gomez, L. Guirado, X. Belmonte, A. Maderuelo, S. Santin, C. Juarez, E. Ars, C. Facundo, J.A. Ballarin, S. Vidal, M.M. Diaz-Encarnacion, Monocyte implication in renal allograft dysfunction, Clin. Exp. Immunol. 175 (2) (2014) 323–331.
[27] Y. Ikezumi, T. Suzuki, T. Yamada, H. Hasegawa, U. Kaneko, M. Hara, T. Yanagihara, D.J. Nikolic-Paterson, A. Saitoh, Alternatively activated macrophages in the pathogenesis of chronic kidney allograft injury, Pediatr. Nephrol. 30 (6) (2015) 1007–1017.
[28] E. Song, H. Zou, Y. Yao, A. Proudfoot, B. Antus, S. Liu, L. Jens, U. Heemann, Early application of Met-RANTES ameliorates chronic allograft nephropathy, Kidney Int. 61 (2) (2002) 676–685.
[29] Q. Yan, H. Jiang, B. Wang, W. Sui, H. Zhou, G. Zou, Expression and significance of RANTES and MCP-1 in renal tissue with chronic renal allograft dysfunction, Transplant. Proc. 48 (6) (2016) 2034.
[30] A.M. Teppo, E. Honkanen, J. Ahonen, C. Gronhagen-Riska, Does increased urinary interleukin-1 receptor antagonist/interleukin-1beta ratio indicate good prognosis in renal transplant recipients? Transplantation 66 (8) (1998) 1009–1014.
[31] B. Afsar, A. Covic, A. Ortiz, R.E. Afsar, M. Kanbay, The Future of IL-1 Targeting in Kidney Disease, Drugs 78 (11) (2018) 1073–1083.
[32] Y. Guan, M.D. Breyer, Peroxisome proliferator-activated receptors (PPARs): novel therapeutic targets in renal disease, Kidney Int. 60 (1) (2001) 14–30.
[33] S. Zafiriou, S.R. Stanners, T.S. Polhill, P. Poronnik, C.A. Pollock, Pioglitazone increases renal tubular cell albumin uptake but limits proinflammatory and fibrotic responses, Kidney Int. 65 (5) (2004) 1647–1653.
[34] K. Isshiki, M. Haneda, D. Koya, S. Maeda, T. Sugimoto, R. Kikkawa, Thiazolidinedione compounds ameliorate glomerular dysfunction independent of their insulin-sensitizing action in diabetic rats, Diabetes 49 (6) (2000) 1022.
[35] S. Zafiriou, S.R. Stanners, S. Saad, T.S. Polhill, P. Poronnik, C.A. Pollock, Pioglitazone inhibits cell growth and reduces matrix production in human kidney fibroblasts, J. Am. Soc. Nephrol. 16 (2005) 638–645.
[36] B.H. Chung, C. Li, B.K. Sun, S.W. Lim, K.O. Ahn, J.H. Yang, Y.H. Choi, K.H. Yoon, A. Sugawara, S. Ito, J. Kim, C.W. Yang, Rosiglitazone Protects against Cyclosporine-Induced Pancreatic and Renal Injury in Rats, Am. J. Transplant. 5 (2005) 1856–1867.
[37] S. Lee, W. Kim, K.P. Kang, S.O. Moon, M.J. Sung, D.H. Kim, H.J. Kim, S.K. Park, Agonist of peroxisome proliferator-activated receptor-gamma, rosiglitazone, reduces renal injury and dysfunction in a murine sepsis model, Nephrol. Dial. Transplant. 20 (6) (2005) 1057–1065.
[38] S. Lee, W. Kim, S.O. Moon, M.J. Sung, D.H. Kim, K.P. Kang, Y.B. Jang, J.E. Lee, K.Y. Jang, S.K. Park, Rosiglitazone ameliorates cisplatin-induced renal injury in mice, Nephrol. Dial. Transplant. 21 (8) (2006) 2096–2105.
[39] K. Baboolal, G.A. Jones, A. Janezic, D.R. Griffiths, W.A. Jurewicz, Molecular and structural consequences of early allograft injury, Kidney Int. 61 (2) (2002) 686–696.
[40] V. Mas, A. Diller, S. Albano, C. Giraudo, T. Alvarellos, J. Sena, P. Massari, B.G. De, Intragraft expression of transforming growth factor-beta 1 by a novel quantitative reverse transcription polymerase chain reaction ELISA in long lasting kidney recipients, Transplantation 70 (4) (2000) 612–616.
[41] Q. Yan, W. Sui, S. Xie, H. Chen, S. Xie, G. Zou, J. Guo, H. Zou, Expression and role of integrin-linked kinase and collagen IV in human renal allografts with interstitial fibrosis and tubular atrophy, Transpl. Immunol. 23 (1) (2010) 1–5.
[42] H. Tang, D. Fan, C.T. Lei, C. Ye, P. Gao, S. Chen, X.F. Meng, H. Su, C. Zhang, MAD2B promotes tubular epithelial-to-mesenchymal transition and renal tubulointerstitial fibrosis via Skp2, J. Mol. Med. 94 (11) (2016) 1297–1307.
[43] T. Vanhove, R. Goldschmeding, D. Kuypers, Kidney Fibrosis: Origins and Interventions, Transplantation 101 (4) (2017) 713–726.
[44] D. Ferenbach, D.C. Kluth, J. Hughes, Inflammatory Cells in Renal Injury and Repair, Semin. Nephrol. 27 (3) (2007) 250–259.
[45] W.D. Park, M.D. Griffin, L.D. Cornell, F.G. Cosio, M.D. Stegall, Fibrosis with inflammation at one year predicts transplant functional decline, J. Am. Soc. Nephrol. 21 (21) (2010) 1987–1997.
[46] F.G. Cosio, M. El Ters, L.D. Cornell, C.A. Schinstock, M.D. Stegall, Changing kidney allograft histology early posttransplant: prognostic implications of 1-year protocol biopsies, Am. J. Transplant. 16 (1) (2016) 194–203.
[47] L.F. Chen, W.C. Greene, Shaping the nuclear action of NF-kappaB, Nat. Rev. Mol. Cell Biol. 5 (5) (2004) 392–401.
[48] G. Tsoulfas, D.A. Geller, NF-kappaB in transplantation: friend or foe? Transpl. Infect. Dis. 3 (4) (2001) 212–219.
[49] C. Guijarro, J. Egido, Transcription factor-kappa B (NF-kappa B) and renal disease, Kidney Int. 59 (2) (2001) 415–424.
[50] I.H. Vos, R. Govers, H.J. Grone, L. Kleij, M. Schurink, R.A. De Weger, R. Goldschmeding, T.J. Rabelink, NFkappaB decoy oligodeoxynucleotides reduce monocyte infiltration in renal allografts, FASEB J. 14 (5) (2000) 815–822.
[51] C.M. Mulders-Manders, M.C. Baas, F.M. Molenaar, A. Simon, Peri- and post-operative treatment with the interleukin-1 receptor antagonist anakinra is Safe in patients undergoing renal transplantation: case series and review of the literature, Front. Pharmacol. 8 (2017) 342.
[52] J. Zegarska, L. Paczek, M. Pawlowska, W. Podrzucki, W. Rowinski, P. Malanowski, M. Wszola, A. Mroz, Quantitative gene expression of TGF-beta1, TNF-alpha, IL-1beta, and IL-6 in the renal artery wall of chronically rejected human renal allografts, Transplant. Proc. 34 (8) (2002) 3176–3179.