Rosiglitazone alleviates injury in rats with adenine-induced chronic kidney disease

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Abstract. Rosiglitazone (ROG) has been shown to exert beneficial effects on glycemic control and renal protection. Circulatory fibroblast growth factor-23 (FGF-23) is a novel independent risk factor for chronic kidney disease (CKD) progression. The current study focused on how ROG impacts on injury of the kidney, and whether FGF-23 is involved in the process. A total of 24 male Sprague Dawley rats, weighing 250-280 g, were divided into four groups (n=6 per group): i) Normal; ii) ROG controls, treated with ROG (10 mg/kg/day); iii) CKD models, treated with adenine (200 mg/kg/day); and iv) ROG treatment, treated with ROG (10 mg/kg/day) and adenine (200 mg/kg/day). The rats were sacrificed after four weeks, and serum, urine and kidney tissues were collected. The data revealed that the serum levels of inorganic phosphate (Pi), intact parathyroid hormone (iPTH) and FGF-23 were significantly higher in the CKD models compared with those in the normal group (P<0.01), while ROG significantly reduced the serum levels of Pi, iPTH and FGF-23 (P<0.01). The ratio of protein/creatine (Cr) in the urine and the serum levels of Cr, blood urea nitrogen and uric acid were significantly increased in the CKD models compared with the normal group (P<0.01), however, ROG significantly reduced these parameters (P<0.05). Furthermore, ROG significantly alleviated the tubule interstitial damage index of the CKD models in comparison with that of the controls. These results indicated that ROG mitigates the lesions of chronic kidney disease induced by adenine, and that the system of Pi-PTH-FGF-23 may contribute to this process. The possible

mechanisms underlying this process require confirmation in future studies.

Introduction

Chronic kidney disease (CKD) is currently considered to be a predominant public health burden and a major cause of morbidity and mortality for diabetic and hypertensive patients worldwide. Recent clinical studies have revealed that persistent proteinuria and serum FGF-23 levels are powerful risk factors for the progression of end-stage renal disease (1-4).

Fibroblast growth factor-23 (FGF-23) is a newly identified member of the FGF family. FGF-23 is secreted from the bone and acts on the kidney to promote urinary inorganic phosphate (Pi) excretion and to suppress vitamin D synthesis, thereby maintaining Pi and calcium homeostasis (5,6). An increasing number of studies have confirmed that the circulatory levels of FGF-23, PTH and Pi are independent risk factors for CKD progression, and are closely correlated with vascular calcification and secondary hyperparathyroidism (7-9).

Proteinuria is a powerful and independent risk factor for kidney disease, cardiovascular disease and all-cause mortality (10). Thus, the reduction in proteinuria invariably translates into protection from a decline in renal function in order to improve the clinical outcome. All studies are committed to reduce proteinuria, improve renal function and extenuate complications in CKD patients (8-10).

Thiazolidinediones (TZDs) are ligands known to bind and activate the nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ). TZDs are used to treat diabetes mellitus, and are known to exert their glucose-lowering effects by reducing insulin resistance through the stimulation of PPAR-γ nuclear receptor. In addition to improving glycemic control, TZDs have been demonstrated to exert beneficial effects on renal and vascular protection (11-15). In the present study, the effect of rosiglitazone (ROG) on proteinuria and renal function in CKD models induced by adenine was analyzed, and the contribution of the Pi-PTH-FGF-23 system was investigated.

Materials and methods

Animal study protocols. This study was conducted according to the guidelines of the National Institute of Health on the care

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and use of laboratory animals. The project was approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University. All rats were housed individually and were provided with normal laboratory chow and tap water *ad libitum* throughout the study. The animals were handled daily to minimize handling stress. Male SD rats (weight, 250-280 g) were obtained from the Animal Center of Sun Yat-Sen University (Guangzhou, China). The SD rats were divided randomly into four groups (n=6 per group): i) Normal group, intragastric administration of saline consistently for four weeks; ii) ROG controls, intragastric administration of rosiglitazone (GlaxoSmithKline plc., Tianjin, China) 10 mg/kg/day consistently for four weeks; iii) CKD model group, intragastric administration of 200 mg/kg/day adenine (Sigma-Aldrich, St. Louis, MO, USA) consistently for four weeks; and iv) ROG treatment group, intragastric admin-istration of ROG (10 mg/kg/day) and adenine (200 mg/kg/day) consistently for four weeks. All rats were sacrificed, and the kidney tissues and blood samples were collected at the end of four weeks. Urine samples of 24-h were collected on the day prior to the rats being sacrificed.

Biochemistry assay. The blood and urine samples from the rats were centrifuged at 1,120 x g for 10 min. The levels of serum creatine (Cr), blood urea nitrogen (BUN), uric acid (UA), calcium, phosphorus, intact parathyroid hormone (iPTH), alkaline phosphatase (ALP) and the 24-h protein/Cr ratio of the urine were tested by an autoanalyzer (Abbott, Chicago, IL, USA) in duplicate.

Enzyme-linked immunosorbent assay (ELISA). The serum levels of FGF-23 were measured by ELISA (FGF-23 ELISA kit; Immutopics, San Clemente, CA, USA) according the manufacturer's instructions.

Hematoxylin and eosin (HE) staining and pathological analysis. The kidneys were fixed in 4% paraformaldehyde overnight and embedded with paraffin for HE staining. Semi-quantitative histological scoring was performed by a pathologist. The scoring of the tubular interstitial damage index (TIDI) used the following criteria: The tubular atrophy and interstitial infiltrates were scored as absent, 0; involving <25% of the biopsy area, 1; involving 26 to 50% of the biopsy area, 2; and involving >50% of the biopsy area, 3. The pres-ence of proteinaceous casts was scored as absent, 0; isolated, 1; present in ≤10% of tubules, 2; and present in >10% of tubules, 3. Tubular dilation was scored as absent, 0; mild, 1; moderate, 2, when associated with flattening of the tubular epithelium; or severe, 3. Tubular dilation was scored as severe, 3, when the diameter exceeded that of the glomeruli; these tubules were often filled with proteinaceous casts.

Statistical analysis. The statistical analysis was conducted using analysis of variance (SPSS 13.0 software: SPSS, Inc., Chicago, IL, USA). P<0.05 was used to indicate a statistically significant difference. The results are expressed as the mean ± SE.

Results

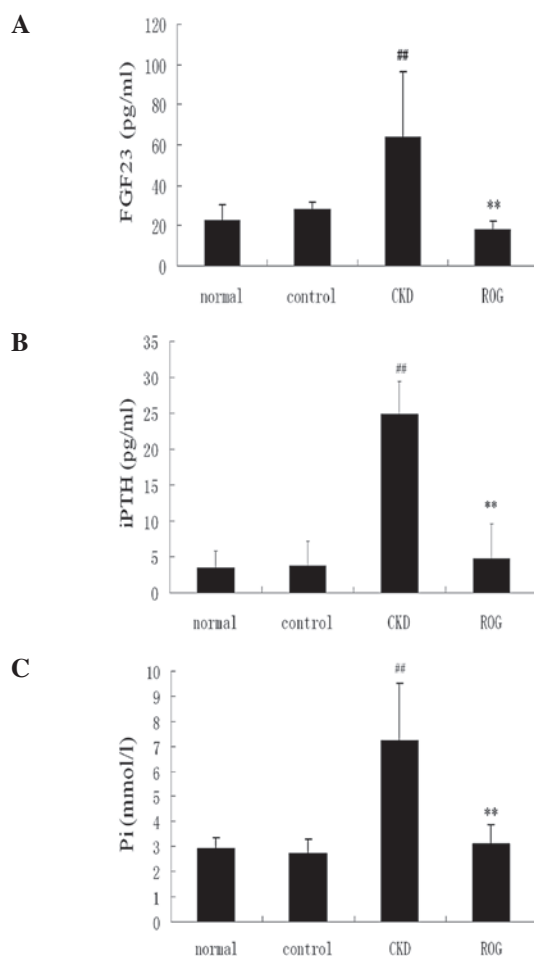*Effect of ROG on the Pi-PTH-FGF-23 system.* The serum levels of FGF-23 were significantly higher in the

Figure 1. (A) Effects of rosiglitazone (ROG) on serum FGF-23 levels among four groups. (B) Effects of ROG on serum PTH levels among four groups. (C) Effects of ROG on serum phosphate (Pi) levels among four groups. #P<0.05 and ##P<0.01 vs. control; *P<0.05 and **P<0.01 vs. model. PTH, para-thyroid hormone; CKD, chronic kidney disease.

CKD model group compared with those of the normal group (64.3±13.2 versus 23.2±7.2 pg/ml; P<0.01). The value of FGF-23 was significantly decreased in the ROG treatment group compared with the CKD model group (17.9±4.5 versus 64.3±13.2 pg/ml; P<0.01). The serum levels of iPTH and Pi were also significantly higher in the CKD model compared with the normal group (P<0.01), but significantly reduced following treatment with ROG in comparison with the CKD model (P<0.01; Fig. 1A-C). However, there were no significant differences in serum calcium and ALP levels among the four groups.

Effect of ROG on proteinuria and renal function. The ratio of protein/Cr of the urine was significantly increased in the CKD models compared with that in the normal group (6,391.4±1,923.0 versus 1,526.1±719.5 mg/g; P<0.01). However, ROG significantly reduced the proteinuria content compared with the CKD models (3,469.75±887.55 versus 6,391.41±1,923.02 mg/g; P<0.01)(Fig. 2A-C). Serum Cr, BUN and UA levels were also significantly increased in the CKD models group compared with the normal group. It is noteworthy that the levels of renal function were significantly improved following treatment with ROG (Fig. 3A-C).

Table I. Comparison of the TIDI in the various groups of the experiment.

Parameter	Normal controls	ROG controls	CKD models	ROG treatment group
TIDI	0.53±0.19	0.61±0.22	9.33±1.74[a]	5.95±0.89[a,b]

[a]P<0.01 vs. control; [b]P<0.05 and vs. model. TIDI, tubular interstitial damage index; ROG, rosiglitazone; CKD, chronic kidney disease.
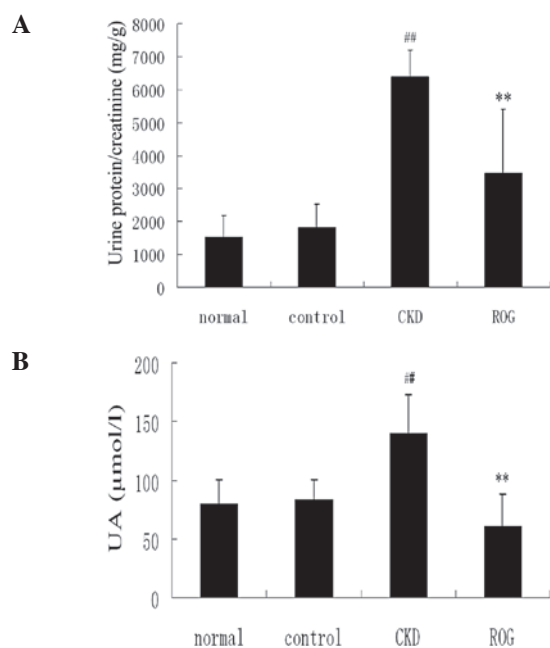

Figure 2. (A) Effects of rosiglitazone (ROG) on the ratio of protein/Cr of the 24-h urine among the four groups. (B) Effects of ROG on the serum uric acid among the four groups. [#]P<0.05 and [##]P<0.01 vs. control; [*]P<0.05 and [**]P<0.01 vs. model. CKD, chronic kidney disease; Cr, creatinine.
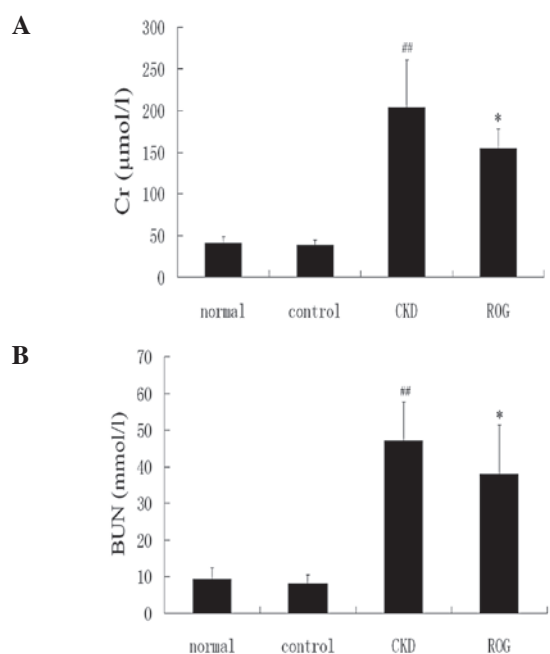

Figure 3. (A) Effects of rosiglitazone (ROG) on serum creatinine (Cr) levels among the four groups. (B) Effects of ROG on serum BUN levels among the four groups. [#]P<0.05 and [##]P<0.01 vs. control; [*]P<0.05 and [**]P<0.01 vs. model. BUN, blood urea nitrogen; CKD, chronic kidney disease.
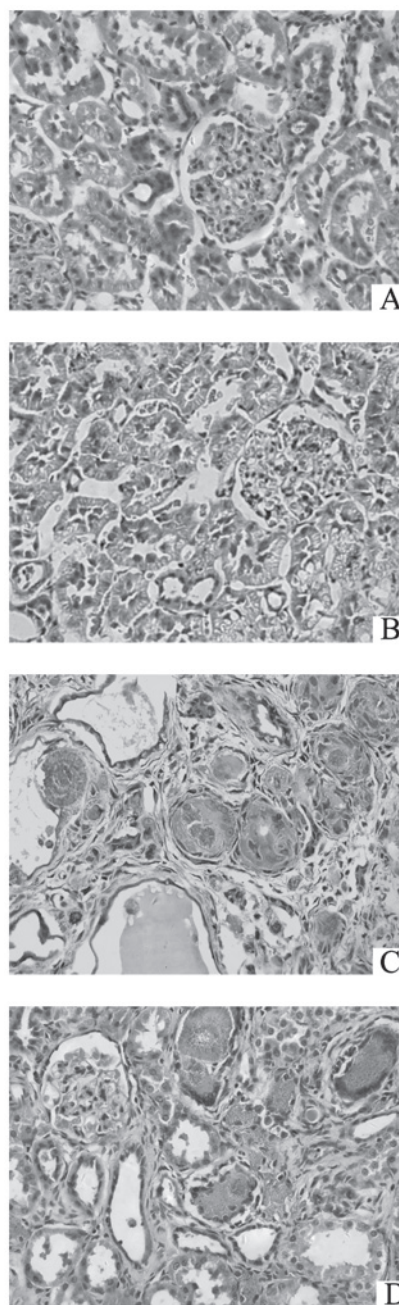

Figure 4. Hematoxylin and eosin staining of the kidney in each group (magnification, x400). (A) Normal; (B) rosiglitazone (ROG) controls; (C) chronic kidney disease models; and (D) ROG treatment.

Renal pathological variation. HE staining demonstrated that the proximal convoluted tubules were swollen, while the distal convoluted tubules and the collecting ducts were expanded with

considerable protein casts and 2,8-dihydroxyadenine crystals in the lumina (Fig. 4C). Monocytes proliferated around the crystals and created nodules, and inflammatory cells infiltrated into the interstitial tissue (Fig. 4C). However, ROG markedly improved the aforementioned pathological lesions, as shown in Fig. 4D. The TIDI values of the CKD models were significantly increased compared with those of the controls (P<0.01), while ROG also significantly improved the TIDI (P<0.05, Table I).

Discussion

In the present study, the CKD rat models were successfully induced with an intragastric administration of adenine, and the serum levels of FGF23, PTH and Pi were confirmed to be consistent with the levels in the lesions of the kidney in this model. Furthermore, this is the first study to demonstrate that ROG was able to attenuate renal damage and metabolic disorders in adenine-induced CKD models. ROG, a PPAR-γ agonist, has been indicated to exert cardiovascular and renal protection through the improvement of anti-inflammation, antiproliferation and antioxidative stress (13-18). These data demonstrated that ROG exerts a polyphenic profit on the cardiovascular and renal system. However, the effect of PPAR-γ on renal proteinuria and renal function are not yet fully understood.

The present study confirmed the effect of ROG on FGF-23 levels and proteinuria, each a risk factor of CKD, thus providing a novel insight into the renoprotective properties of the drug. The results of this study demonstrated that the serum FGF-23, PTH and Pi levels were markedly increased in adenine-induced CKD rats, but that ROG was able to effectively attenuate the augmentation. FGF-23 is part of a previously unrecognized hormonal bone-PTH-kidney axis. Mineral and bone metabolism may be viewed as a complex system in which three hormones (PTH, calcitriol and FGF-23) act on three key organs (the kidneys, intestines and bones) to adjust plasma, calcium and phosphorus concentrations (19). Mineral disorders, including an increase in FGF-23 and PTH, and hyperphosphatemia, are independent risk factors in CKD progression and mortality (3,20,21). The present data revealed that ROG reduced the serum levels of FGF-23, PTH and phosphorus in the CKD rats, indicating that ROG may have a beneficial effect on the kidney through the attenuation of mineral disorders in adenine-induced CKD models.

It was also observed that the intervention with ROG decreased the proteinuria and protected the renal function in the adenine-induced CKD models. The present results were consistent with other experimental studies that demonstrated that TZDs are beneficial for renal and cardiovascular protection (13,18,22,23). ROG intervention attenuated hyperuricemia, which is generally acknowledged as an independent risk factor of CKD. Thus, the current study revealed that ROG, a PPAR-γ agonist may decrease proteinuria and attenuate metabolic disorders, including mineral disorders and hyperuricemia, in adenine-induced CKD models. Therefore, a novel renoprotective effect of TZDs may be indicated based on the present study. Further studies are required to confirm these findings.

Acknowledgements

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