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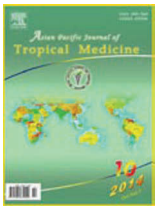

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Protective effect of pioglitazone on kidney injury in diabetic rats

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ABSTRACT

Objective: To investigate the protective effect of pioglitazone on kidney injury in diabetic rat model and its mechanisms. **Methods:** Forty healthy Sprague Dawley rats were selected and randomly divided into five groups, with 8 rats in each group. Group A served as control group and were administered with sterile citrate buffer (*i.p.*) as placebo. Groups B, C, D and E rats were injected (*i.p.*) with streptozotocin to induce type Ⅰ diabetes. Diabetic rats in Group B were intragastrically administered with sterile saline solution alone. Groups C, D and E rats were intragastrically given pioglitazone hydrochloride suspension at doses of 10, 20, 30 mg/kg per day, respectively. After eight weeks of treatment, all rats were anesthetized and blood was withdrawn from the abdominal aortic for detection of hemoglobin A_{1c}, serum creatinine (SCr) and blood urea nitrogen (BUN) levels. Rats were then sacrificed and the left kidney was excised for calculation of kidney hypertrophy index (KHI), observation of renal pathological changes using light microscope and electron microscope. Mean glomerular cross-sectional areas (MGA), mean glomerular volume (MGV), glomerular basement membrane thickness and foot process fusion ratio were calculated. RT-PCR was employed for detection of podocalyxin (PCX) protein expression. **Results:** Results showed that levels of hemoglobin A_{1c}, BUN, SCr in Groups B, C, D and E rats were significantly higher than those in Group A ($P<0.05$), while BUN and SCr levels in rats of Groups C, D and E were significantly lower than those in Group B ($P<0.05$). KHI, MGA and MGV levels were significantly higher in Groups B, C, D and E rats than those in Group A ($P<0.05$); KHI and MGA levels in Group B rats were significantly higher than those in Groups C, D and E ($P<0.05$) and MGV in Groups D and E was significantly lower than that in Groups B and C ($P<0.05$). Histology study showed normal glomerulus structure, morphology, volume, endothelial cells and mesangial cells as well as clear glomerular capillary in Group A rats. Renal mesangial matrix proliferation and expansion of glomerulus cavities in Groups B, C, D and E were observed. However, damage degree in Groups C, D and E were more moderate than that in Group B. **Conclusions:** Pioglitazone can reduce kidney damage in diabetic rats, which may be attributed to its role in increasing glomerular PCX protein expression and inhibiting urinary excretion of PCX, and its effect is dose dependent.

1. Introduction

With the changes of living environments and conditions, the morbidity of diabetes mellitus in the world shows a trend of increase year by year[1]. According to previous reports[2-5], the morbidity of adult diabetes around the world has been as high as 9.7%. Diabetic nephropathy (DN) is a major chronic complications of diabetes and has become the main cause of end-stage renal disease[6]. The pathogenesis of DN is complex; it is a result of the combined action of multiple factors. The main pathological changes are expansion of mesangial area, thickening of glomerular basement membrane with or without fibrosis of tubulointerstitial as well as the glomerular sclerosis in the end. If not treated timely, DN could finally progress to end-stage renal disease which would seriously affect the living quality of the patients[7]. Previous studies have reported that pioglitazone can reduce DN kidney damage through improving glucolipid metabolism, relieving insulin resistance, and some other non-hypoglycemic action mechanisms[8]. With the aim of investigating the protective effect and mechanism of pioglitazone against kidney damage in diabetic rats, streptozotocin induced type Ⅰ diabetic Sprague Dawley rats were selected and treated with different doses of pioglitazone. The protective effect and

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possible mechanism of pioglitazone on kidney damage in diabetic rats were observed so as to provide an experimental basis for the use of pioglitazone in the clinical prevention and treatment of DN.

2. Materials and methods

2.1. Animals

Forty healthy male adult Sprague Dawley rats with 8 weeks of age and weight of 250-300 g were selected from the animal center, Hainan Medical University Affiliated Hospital, Haikou, Hainan, China. Every four rats were kept in one cage with food and water *ad libitum* under condition of relative humidity of 40%-60% and temperature of 20-30 ℃. Ventilation and lighting were provided regularly. During the experiment, animals were treated strictly according to the rules in the Regulations for the Administration of Affairs Concerning Experimental Animals.

2.2. Apparatuses and reagents

Apparatuses like Ultra 2 full-automatic glycosylated hemoglobin analyser, glucometer, EPS-600 electrophoresis apparatus (Tanon Science & Technology Co., Ltd., Shanghai, China), Biosens 805 gel documentation system (Shanghai Bio-Tech Co.,Ltd, Shanghai, China); Micropublisher 5.0 imaging system (Qimaging Company, Canada), HT1 microplate reader, BX-41 biological microscope and JEM-1230 transmission electron microscope (JEOL Company, Japan) were used in the experiment. Reagents such as streptozotocin (Sigma, USA); insulin glargine and pioglitazone hydrochloride tablets (Jiangsu Hengrui Medicine Co. Ltd., China); chloral hydrate (Sanli Company, Shenzhen); urinary albumin (UAlb) kits, ELISA test kits and PCR kit (R&D, USA), triglyceride, total cholesterol, high density lipoprotein cholesterol, low-density lipoprotein cholesterol and creatinine reagent kits (Bioss, Beijing, China); Triton X-100, podocalyxin (PCX) polyclonal antibody, Trizol and diethylpyrocarbonate (Gibco, USA) were used.

2.3. Diabetes mellitus modeling

Diabetes mellitus rat model was constructed by injection (*i.p.*) of streptozotocin. Streptozotocin (65.0 mg/kg) was injected into the 12-hour fasting rats. After 72 h, blood was withdrawn from the tail and blood glucose levels were measured. Modeling was considered to be successful if blood glucose level was higher than 16.7 mmol/L accompanied with dieresis and polydipsia in rats.

2.4. Methods

Forty Sprague Dawley rats were randomly divided into five groups, with 8 rats in each group. Group A served as normal control group without induction of diabetes and were administered with sterile citrate buffer (*i.p.*) as placebo alone. Diabetes mellitus model was constructed in Groups B, C, D and E rats. Group B served as diabetes mellitus group. After diabetes mellitus modeling, Group B rats were intragastrically administered with sterile saline solution alone. Diabetic rats in Groups C, D and E were intragastrically given pioglitazone hydrochloride suspension for eight weeks at doses of 10, 20, 30 mg/kg per day, respectively. During the study, if blood glucose levels were higher than 33.3 mmol/L, insulin glargine (0.5 IU) were injected (*i.p.*) for treatment. The blood glucose levels were adjusted to keep it at the level of 20-30 mmol/L.

2.5. Biochemical indices

After eight weeks of treatment, all rats were anesthetized and blood was withdrawn from the abdominal aorta for detection of hemoglobin A_{1c} (HbA_{1c}), serum creatinine (SCr) and blood urea nitrogen (BUN) levels.

2.6. Histomorphological observation

Rats were then sacrificed after blood drawing and the left kidney was excised for calculation of kidney hypertrophy index (KHI), observation of renal pathological changes under light microscope and electron microscope. Mean glomerular cross-sectional areas (MGA), mean glomerular volume (MGV), glomerular basement membrane thickness (GBMT) and foot process fusion ratio (FPFR) were calculated. RT-PCR was employed for detection of PCX protein expression.

2.7. Statistical analysis

Data were analyzed using SPSS 19.0 statistic software and expressed as mean±SD. Results were compared using One-way ANOVA and then tested using Q-test. *Chi*-square test was employed for qualitative data analysis. P value less than 0.05 was considered as statistically significant difference.

3. Results

3.1. Comparison of HbA_{1c}, SCr and BUN levels in different groups

Results showed that levels of HbA_{1c}, BUN, SCr in Groups B, C, D and E rats were significantly higher than those in Group A ($P<0.05$). BUN and SCr levels in rats of Groups C, D and E were significantly lower than those in Group B ($P<0.05$). However, there was no significant difference among Groups C, D and E ($P>0.05$) (Table 1).

Table 1
Comparison of HbA_{1c}, SCr and BUN levels among different groups after treatment.

Groups	n	HbA_{1c} (%)	BUN (mmol/L)	SCr (μ mol/L)
Group A	8	3.89±0.56	2.95±0.50	55.09±8.05
Group B	8	11.12±1.55*	10.00±1.53*	116.49±16.21*
Group C	8	10.56±1.23*	8.11±1.03*#	93.17±13.07*#
Group D	8	10.55±1.42*	7.79±1.29*#	93.66±17.29*#
Group E	8	10.50±1.09*	7.15±0.89*#	98.29±13.65*#

*$P<0.01$ compared with Group A; #$P<0.05$ compared with Group B.

3.2. Comparison of KHI, MGA, MGV, GBMT and FPFR in different groups

KHI, MGA and MGV levels were significantly higher in Groups B, C, D and E rats than those in Group A ($P<0.05$);

KHI and MGA levels in Group B rats were significantly higher than those in Groups C, D and E (P<0.05) and MGV in Groups D and E was significantly lower than that in Groups B and C (P<0.05). It was observed from the electron microscope that glomerular capillary basement membranes in Group B rats were irregular thickening and blurring with foot process fusion and damage, and both GBMT and FPFR in Group B rats were significantly higher than those in Group A (P<0.05). While GBMT and FPFR were significantly lower in Groups C, D and E than those in Group B, but all parameters were higher than those in Group A (P<0.05). Compared with Group C, GBMT and FPFR in Groups D and E were significantly lower (P<0.05) (Table 2).

Table 2

Comparison of KHI, MGA, MGV, GBMT and FPFR in different groups.

Groups	n	KHI ($\times10^{-3}$)	MGA ($\times10^{3}$ μm^2)	MGV ($\times10^{4}$ μm^3)	GBMT (nm)	FPFR
Group A	8	3.06±0.44	6.02±1.10	59.19±16.23	101.78±15.71	0.03±0.01
Group B	8	6.10±0.63*	9.39±1.36*	102.21±22.37*	294.06±29.32*	0.86±0.05*
Group C	8	5.28±0.74$^{*\triangle}$	8.21±1.08$^{*\triangle}$	92.11±17.95*	210.42±16.84$^{*\triangle}$	0.72±0.05$^{*\triangle}$
Group D	8	4.73±0.37$^{*\triangle\#}$	7.33±0.78$^{*\triangle\#}$	82.70±14.53$^{*\triangle}$	132.02±17.99$^{*\triangle\#}$	0.50±0.06$^{*\triangle\#}$
Group E	8	4.71±0.37$^{*\triangle\#}$	7.30±1.17$^{*\triangle\#}$	78.78±18.09$^{*\triangle}$	129.65±18.34$^{*\triangle\#}$	0.46±0.05$^{*\triangle\#}$

*: P<0.05 compared with Group A; $^{\triangle}$: P<0.05 compared with Group B; $^{\#}$: P<0.05 compared with Group C.

3.3. Histological changes in different groups

Histological study showed normal glomerulus structure, morphology, volume, endothelial cells and mesangial cells as well as clear glomerular capillary loops in Group A rats. Renal mesangial matrix proliferation and expansion of glomerulus cavities in Groups of B, C, D and E were observed. However, damage degree in Groups C, D and E were more moderate than that in Group B (Figure 1).

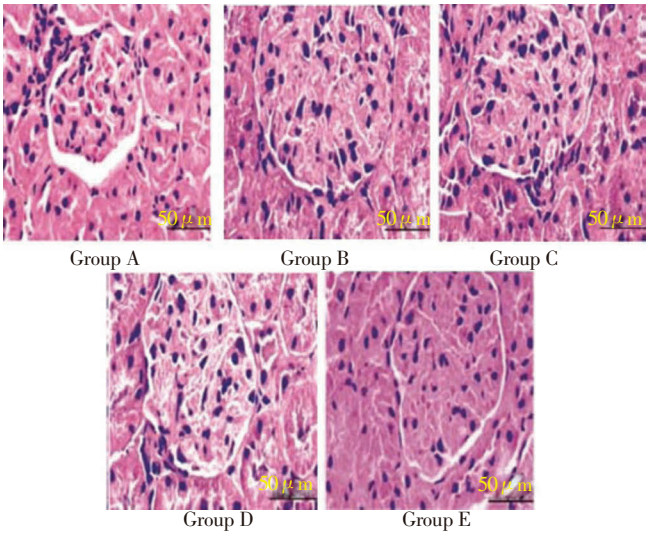

Figure 1. Renal histological changes in different groups (HE, ×400).

3.4. Expression of PCX protein in renal tissues of different groups

In renal tissues of Group A rats, PCX protein was present as claybank granule and positively distributed along with glomerular peripheral capillary loops. While PCX protein in renal tissues of Groups B, C, D and E rats was characterized by inhomogeneous distribution, some even disappeared. However, positive staining of PCX protein in Groups C, D, E rats was significantly more than that in Group B (Figure 2).

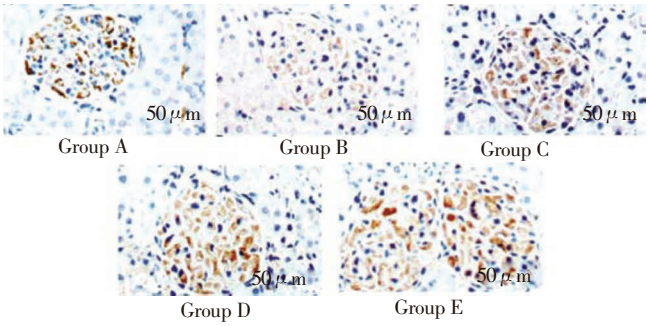

Figure 2. Expression of PCX protein in renal tissues of different groups (×400, Elivision).

4. Discussion

Diabetes mellitus is a common clinical disease with an increasing morbidity around the world. DN is a major complication of diabetes mellitus and its main pathological changes include expansion of renal mesangial area and damage of glomerular, which is a leading cause of end-stage renal disease, seriously influencing physical and mental health of the patients[9]. The pathogenesis of DN is complex. Clinically, it is considered that sustained hyperglycemia and genetic predisposition are on the basis of morbidity of DN[10]. Pioglitazone is a thiazolidinediones anti-diabetic drug. It is an insulin sensitizer and its mechanism of action is associated with the presence of insulin. Pioglitazone can reduce the insulin resistance in peripheral tissue and liver, increase the processing of glucose that is dependent on insulin, and reduce the output of glycogen. Unlike sulfonylurea, pioglitazone is not a promoter for insulin secretion. It works through highly selectively activating the activities of peroxisome proliferator-activated receptor- γ 1. The activation of peroxisome proliferator-activated receptor- γ 1 can adjust transcription of many insulin related genes which are in charge of controlling the metabolism of glucose and lipid[11-13]. According to previous studies[8], pioglitazone can alleviate kidney disease through some mechanisms other than hypoglycemic activity. The present study aimed to investigate the protective effect of pioglitazone against kidney injury in diabetic rats and its mechanisms so as to provide an experimental basis for the clinical use of pioglitazone in prevention and treatment of DN.

Podocytes are glomerulus visceral epithelial cells. Its structure includes the primary process, cell body and foot process which are the most vulnerable parts when suffered from DN[14]. According to previous studies[15,16], structure and function changes of podocytes could occur at the early stage of glomerular injury in diabetic, indicating the close relationship between podocyte damage and onset of DN. Another study confirmed that there could be abnormity in the podocytes related protein and (or) the number of podocytes during the onset of DN[17]. In the present study, GBMT and FPFR in Groups B, C, D and E rats were significantly higher than those in Group A (P<0.05), which also confirmed that the podocyte damage occurred in DN; while compared with Group B, GBMT and FPFR in Groups

C, D and E rats were significantly lower but higher than Group A (P<0.05); glomerular GBMT and FPFR in Groups D, E rats were lower than those of Group C (P<0.05), indicating that pioglitazone has remarkable protective effect on the kidney in a dose dependent manner. Results also showed that compared with Group A, HbA_{1c}, BUN and SCr levels in Groups B, C, D and E rats were significantly higher (P<0.05); while BUN and SCr levels are significantly lower in Groups C, D and E than those in Group B (P<0.05), indicating that kidney treated with pioglitazone possessed a stronger toxin scavenging ability than that in Group B and pioglitazone had a protective effect on DN rat kidney. PCX, a member of CD34 family, is usually expressed in hematopoietic stem cells, kidney podocytes and vascular endothelial cell[18,19]. PCX itself carries negative charges, making it have exclusive effect by avoiding adhesion of foot processes among adjacent podocytes, thus, maintaining the open status of glomerular filtration membrane[20,21]. In renal tissues of Group A rats, PCX protein was present as claybank granule and positively distributed along with glomerular peripheral capillary loops. While PCX protein in renal tissues of rats in Group B, C, D and E was characterized under inhomogeneous distribution, some even disappeared. However, positive staining of PCX protein in rats of Groups C, D, E was significantly more than that in Group B, indicating that treatment with pioglitazone at the early stage can obviously inhibit the down regulation of PCX expression and excretion of PCX along with urinary, so as to protect the renal function in DN. In the histological observation, it was showed that the degree of glomerular damage in rats of Groups C, D and E was slighter than that in Group B, which also confirmed that pioglitazone can reduce glomerular injury in diabetic rat and protect renal function.

The results in the present study showed that pioglitazone remarkably reduced kidney damage in diabetic rats, which may be attributed to its role in increasing glomerular PCX protein expression and inhibiting urinary excretion of PCX.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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