

Original Article

Ginseng saponins Rg1's effect on the expression of tumor necrosis factor alpha, monocyte chemotactic factor protein-1 in diabetic nephropathy rats

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Abstract: Diabetic nephropathy (DN) is a common complication of diabetes mellitus. Inflammation mechanism is a key factor for DN sustainable development. Monocyte chemotactic protein 1 (MCP-1) is a main factor of DN inflammatory infiltration, which generates a large amount of tumor necrosis factor α (TNF- α). Ginsenoside Rg1 can reduce 24 h urine protein and blood β 2 microglobulin levels. However, the role of ginsenoside Rg1 in intervening inflammation and improving renal function in DN is still unclear. This study explored the impact of ginsenoside Rg1 on TNF- α and MCP-1 expression in diabetic nephropathy rat. 80 male rats were equally randomly divided into normal control, diabetes model group, ginsenoside Rg1 group, and irbesartan (ARB) group. Diabetes model was established by streptozotocin (STZ) abdominal injection at 65 mg/kg. Blood glucose, 24 h urinary protein, and serum creatinine level were detected. MCP-1 and serum TNF- α levels were tested by ELISA. MCP-1 and TNF- α mRNA expression were determined by Real time PCR. Compared with model group, 24 h urine protein and serum creatinine levels significantly reduced in treatment group ($P < 0.05$). Ginsenoside Rg1 group and ARB group presented declined MCP-1 and TNF- α levels than model group ($P < 0.01$). Ginsenoside Rg1 can improve renal function in DN, which facilitates renal pathological damage repair and renal protection.

Keywords: Diabetic nephropathy, ginsenoside Rg1, MCP-1, TNF- α

Introduction

Diabetic nephropathy (DN) is the most common complication of diabetes. Its pathogenesis is complex and various. It mainly damages microvessel with high mortality and disability rate [1]. Research about DN pathogenesis is relatively mature, mainly including hemodynamic disorder, glycometabolism disorder, oxidative stress, insulin resistance, and immuno-inflammatory response, etc. Immuno-inflammatory response can lead to diabetes internal environment imbalance, resulting in renal cell damage and even glomerular sclerosis [2]. Inflammatory cells such as monocyte/macrophage inflammatory infiltration are the main factors of DN [3]. It was found that the main chemokine in the monocyte/macrophage was MCP-1. It can not only trigger inflammatory response, and can accelerate glomerular mesangial cells proliferation, leading to renal cells generate a large amount of matrixes. Matrix deposition speeds up glomerular sclerosis. Together with

tissue inflammatory infiltration generated tumor necrosis factor α (TNF- α), it further accelerates inflammatory medium activation and generation [4]. Ginsenoside Rg1 is one of the main effective monomer compositions of panax notoginsenoside, featured as reducing 24 h urine protein and serum β 2 microglobulin so as to repair the kidney damage. Early study discovered that Rg1 can obviously repair the renal interstitial fibrosis in rats [5]. However, the role of ginsenoside Rg1 in intervening inflammation and improving renal function in DN is still unclear. This study investigated the impact of ginsenoside Rg1 on TNF- α and MCP-1 expression in DN rat to provide reference for clinical treatment.

Materials and methods

Experimental animals

Healthy male SD rats weighted 210~280 g were provided by Henan laboratory animal cen-

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Table 1. Primer sequence

Gene	Sequence	Length (bp)
MCP-1	Forward, 5'-CACGTCGTAGCAAACCACCAA-3'	113
	Reverse, 5'-GTTGGTTGTCTTTGAGATCCAT-3'	
TNF- α	Forward, 5'-CACGTCGTAGCAAACCACCAA-3'	100
	Reverse, 5'-GTTGGTTGTCTTTGAGATCCAT-3'	
GAPDH	Forward, 5'-TGACATCAAGAAGGTGGTGA-3'	117
	Reverse, 5'-TCATACCAGGAAATGAGCTT-3'	

Index detection

HE staining: The rats were anesthetized by 10% chloral hydrate intraperitoneal injection at 0.3 mL/100 g and euthanized. Kidney tissue was put out and fixed in 40 g/L paraformaldehyde solution. Then the tissue was routinely treated by embedding, sectioning, and staining.

Biochemical index detection

24 h urine was collected on the last day of eight weeks' experiment to detect 24 h urine protein and Cr value. At 12 h after fasting, venous blood was collected to test fasting blood glucose.

MCP-1 and TNF- α detection

Serum MCP-1 and TNF- α levels were detected according to ELISA kit instruction. Real time PCR was applied to test TNF- α and MCP-1 mRNA expression. Renal tissue RNA was extracted by Trizol and tested by ultraviolet spectrophotometer. Then the RNA was reverse transcribed to cDNA. Real time PCR consisted 94°C for 1 min, followed by 50 cycles of 94°C for 10 s, 54°C for 10 s, and 72°C for 10 s. GAPDH was selected as internal reference. Relative gene expression was semiquantitative analyzed by $2^{-\Delta\Delta Ct}$ method. $2^{-\Delta\Delta Ct}$ = gene copy number in test group/gene copy number in control [7]. The primer sequence of TNF- α , MCP-1, and GAPDH was listed in **Table 1**.

Statistical analysis

SPSS 15.0 was applied for data analysis. Measurement data were presented as mean \pm standard deviation. Data comparison was performed by one-way ANOVA. $P < 0.05$ was considered as statistical significance.

Results

Pathological changes

HE staining revealed that no renal tissue pathological damage was observed in normal control. The kidney in model group showed a small percentage of glomerular enlargement, renal tissue basement membrane thickening, and inflammatory cells infiltration, leading to capillaries diameter decrease, renal tubular epithelial cell cytoplasm reduction, and mild tissue edema. Compared with ARB group, ginsenoside

ter (license: SCXK-2015-0004). The rats were breed at room temperature (20~25°C) and suitable relative humidity (50~60%). All disposal on animal accorded with ethics standards.

Reagents and instruments

Methoxy polyethylene glycol-superoxide was bought from Horizon Chemical Industry. In situ apoptosis detection kit was purchased from ZSbio. Pressure sensor was from Gloud. Ginsenoside Rg1 was from Nanjing plant industry co., LTD. Irbesartan (50 mg/piece, H20000-513) was got from Jiangsu Heng Rui medical co., LTD. MCP-1 and TNF- α ELISA kits were purchased from BIOSOURCE. Type 7200 automatic analyzer was bought from Shanghai Shida Biotechnology Company. Trizol, RNA extraction kit, and reverse transcription kits were bought from Gloud. Brilliant SYBR Green QPCR mastermix was purchased from Brilliant. Glucometer was from Shanghai Nioda Biotechnology Company. Real-time PCR instrument was purchase from Shanghai Reese biological technology co., LTD. Transmission electron microscopy was purchased from Nanjing Conrad biological technology co., LTD.

Modeling

Animal model establishment and grouping [6]: 80 SD rats were randomly equally divided into normal control, model group, ginsenoside Rg1 group, and irbesartan group. Diabetes model was established by streptozotocin (STZ) abdominal injection at 65 mg/kg in addition to normal control. Successful DN model criteria: continuous STZ intraperitoneal injection for 1 week, fasting blood glucose > 16.7 mmol/L, and glycosuria 3+. After modeling, ginsenoside Rg1 (50 mg/kg/d) was injected to stomach in ginsenoside Rg1 group, while irbesartan (50 mg/kg/d) was gave to ARB group. The rats in control and model group received equal doses of distilled water. The total experimental period was eight weeks.

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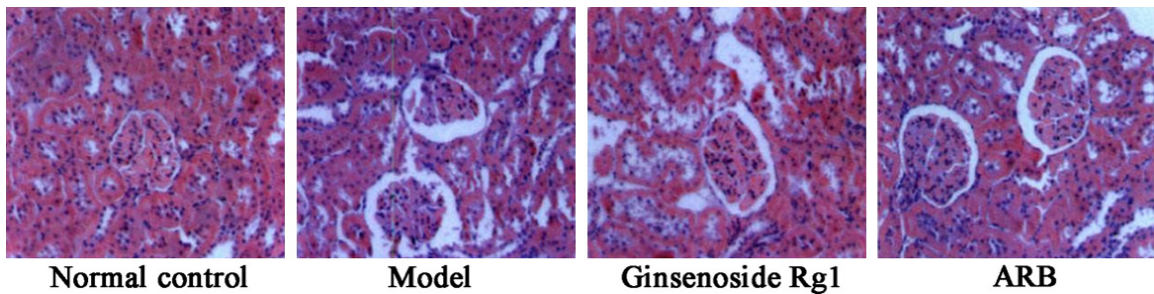


Figure 1. Renal tissue pathological changes, HE \times 400.

Table 2. Biochemical index comparison

Group	Blood glucose (mmol/L)	24 h urine protein (mg/24 h)	Cr (μ mol/L)
Normal control	5.16 \pm 0.65	1.54 \pm 0.04	53.22 \pm 4.54
Model group	24.98 \pm 1.85*	9.12 \pm 0.56*	94.71 \pm 8.32*
Ginsenoside Rg1 group	22.43 \pm 1.21	5.21 \pm 0.32 ^a	56.32 \pm 4.32 ^a
ARB group	22.12 \pm 1.32	4.21 \pm 0.43 ^a	54.43 \pm 4.12 ^a

*P < 0.05, compared with normal control; ^aP < 0.05, compared with model group.

Rg1 group presented no obvious renal tissue pathological changes. Two groups appeared only a small amount of capillary loops basement membrane slightly thickening and mesangial area broaden. However, inflammatory cell number declined and no significant pathological changes were observed in renal tubules and interstitial (**Figure 1**).

Biochemical index changes

Compared with normal control, blood glucose, 24 h urine protein, and Cr value obviously increased in model group, ginsenoside Rg1 group, and ARB group. Compared with model group, ginsenoside Rg1 group and ARB group presented significantly declined 24 h urine protein level and Cr level but not blood glucose. No statistical differences about 24 h urine protein and Cr value were observed between ginsenoside Rg1 group and ARB group (P > 0.05) (**Table 2**).

Ginsenoside Rg1 impact on MCP-1 and TNF- α mRNA and protein expression

MCP-1 mRNA expression in renal tissue from model group significantly elevated compared with normal control (P < 0.05). Ginsenoside Rg1 group and ARB group showed obviously

reduced MCP-1 mRNA level compared with model group (P < 0.05). No statistical difference of MCP-1 mRNA was observed between ginsenoside Rg1 group and ARB group (P > 0.05). Consistent with the changes of MCP-1 mRNA expression, IHC analysis also showed reduced expression of MCP-1 protein expression in Ginsenoside Rg1 group and ARB group (**Figure 2**).

TNF- α mRNA expression in renal tissue from model group significantly increased compared with normal control (P < 0.05). Ginsenoside Rg1 group and ARB group presented obviously declined TNF- α mRNA level compared with model group (P < 0.05). Consistently, IHC analysis also demonstrated reduced expression of TNF- α protein expression in Ginsenoside Rg1 group and ARB group (**Figure 3**). No statistical difference of TNF- α mRNA was found between ginsenoside Rg1 group and ARB group (P > 0.05) (**Table 3**).

Ginsenoside Rg1 impact on MCP-1 and TNF- α protein expression

Serum MCP-1 level in renal tissue from model group significantly elevated compared with normal control (P < 0.05). Ginsenoside Rg1 group and ARB group showed obviously reduced serum MCP-1 level compared with model group (P < 0.05). No statistical difference of serum MCP-1 level was observed between ginsenoside Rg1 group and ARB group (P > 0.05).

Serum TNF- α expression in renal tissue from model group significantly increased compared with normal control (P < 0.05). Ginsenoside Rg1 group and ARB group presented obviously declined serum TNF- α level compared with

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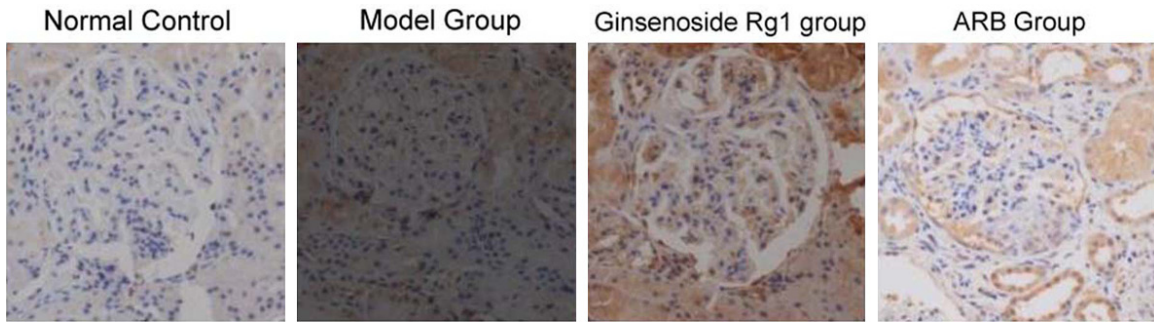


Figure 2. IHC analysis of MCP-1 expression in kidney (x 400).

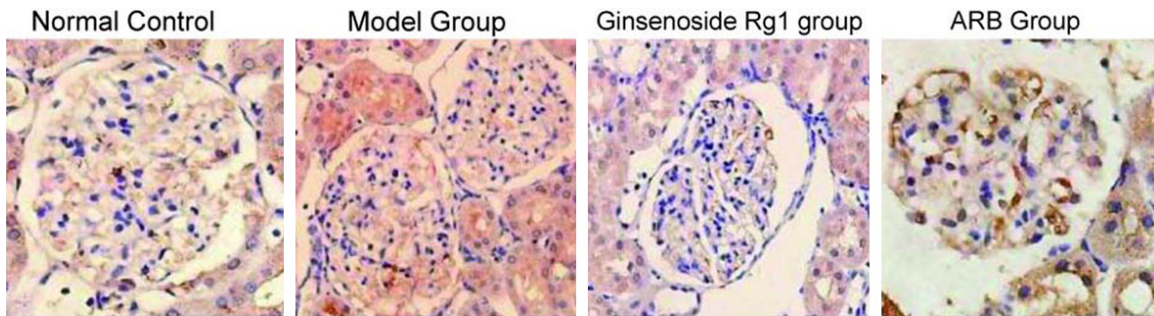


Figure 3. IHC analysis of TNF- α expression in kidney (x 400).

Table 3. MCP-1 and TNF- α mRNA level comparison

Group	MCP-1 mRNA (median)	TNF- α mRNA (median)
Normal control	1	1
Model group	6.5*	6*
Ginsenoside Rg1 group	3.5* Δ	3.5* Δ
ARB group	2.5* Δ	2.5* Δ

*P < 0.05, compared with normal control; Δ P < 0.05, compared with model group.

Table 4. Serum MCP-1 and TNF- α levels comparison

Group	MCP-1 (μ g/L)	TNF- α (μ g/L)
Normal control	3.65 \pm 0.31	6.12 \pm 0.23
Model group	14.21 \pm 0.67*	14.32 \pm 0.76*
Ginsenoside Rg1 group	8.51 \pm 0.54* Δ	6.98 \pm 0.45* Δ
ARB group	6.21 \pm 0.35* Δ	6.32 \pm 0.34* Δ

*P < 0.05, compared with normal control; Δ P < 0.05, compared with model group.

model group (P < 0.05). No statistical difference of serum TNF- α level was found between ginsenoside Rg1 group and ARB group (P > 0.05) (Table 4).

Discussion

Study revealed that STZ intraperitoneal injection at 65 mg/kg may cause blood glucose and Cr value elevation, even glomerular pathological changes (including glomerular basement membrane hyperplasia, mesangial matrix production, and inflammatory cells infiltrating growth), etc. These results confirmed our successful DN model [8]. Experimental results demonstrated that irbesartan can decrease 24 h urine protein and Cr value in diabetes rats, indicating that irbesartan can improve renal damage in DN rats independent of controlling blood glucose. Thus, it was selected as positive control.

As a monomer composition, ginsenoside Rg1 is featured as protecting renal function through reducing 24 h urine protein and blood β 2 microglobulin [9]. Our results showed that compared with model group, ginsenoside Rg1 treatment obviously declined 24 h urine protein and blood Cr value, suggesting that it can significantly improve STZ induced DN pathological damage. Therefore, we speculated that ginsenoside Rg1 can improve DN damage.

Study found that proteinuria was a common clinical manifestation in renal disease. For DN study, proteinuria can not only be treated as a clinical indicator, also as the main indicator for kidney disease progress [10]. Podocytes in kidney appeared abnormal morphology, leading to large amount of proteinuria generation that has correlation with glomerular pathological changes [11]. Our experimental results showed that renal tissue only appeared slight capillary loops basement membrane thickening and mesangial area broaden in ginsenoside Rg1 group. However, the number of infiltrated inflammatory cells decreased, and no obvious pathological changes were observed in renal tubules and interstitial in renal tissue after ginsenoside Rg1 treatment. Recent clinical studies confirmed that inflammatory response played a key role in diabetes disease. Inflammatory cells, such as monocyte/macrophage, can infiltrate kidney part, and chemokines generation can speed up DN progress [12-14]. At present, it was found the main chemotactic factor in monocyte/macrophage is MCP-1. It not only can trigger inflammatory response, but also can accelerate glomerular mesangial cells proliferation with numerous matrixes, which further facilitates glomerular sclerosis [15, 16]. Following inflammation infiltration generated TNF- α , it further aggregates inflammatory mediators' production [17, 18].

Our study indicated that MCP-1 and TNF- α obviously increased at gene and protein level, which is consistent with previous results [19, 20]. In addition, we found that though blood glucose level reduced in both ginsenoside Rg1 and ARB group, it was lack of statistical difference. On the other hand, 24 h urine protein and Cr value obviously declined in two groups, suggesting inflammation response and inflammatory factors played important roles in DN. Ginsenoside Rg1 treatment significantly decreased MCP-1 and TNF- α , alleviated renal pathological changes, and delayed renal disease progress.

In conclusion, ginsenoside Rg1 can improve renal function and damage in DN rat. It can protect kidney by repairing renal pathological damage.

Disclosure of conflict of interest

None.

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