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Short Communication

Effects of Tetrahydroxystilbene Glucoside on VEGF and MMP-9 Protein Expression in Kidney Tissues of Rats with Diabetic Nephropathy

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XL designed the study, performed the experiments and wrote the paper. ZL performed the experiments and reviewed the manuscript.

Key words
Tetrahydroxystilbene glucoside (TSG), Diabetic nephropathy(DN), Vascular endothelial growth factor (VEGF), Matrix metalloproteinase (MMP-) 9, Kidney tissues

ABSTRACT

The purpose of this study was to investigate intervention effects of tetrahydroxystilbene glucoside (TSG) in diabetic nephropathy (DN) rats and analyze its action on vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP-) 9 protein expression in kidney tissues, in order to reveal the mechanism of TSG. After 12 weeks of administration, compared with those in the DN modeling group, 24 h urine protein, serum creatinine (Scr), blood urea nitrogen (BUN), blood uric acid (UA), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) significantly reduced in rats treated with TSG (20 mg/kg) ($P<0.05$), blood total protein (TP) and albumin (ALB) significantly increased ($P<0.05$), VEGF expression significantly reduced ($P<0.05$) and MMP-9 expression significantly increased ($P<0.05$). The present study results showed that TSG could reduce proteinuria levels, slow down the progression of DN, and have a certain protective effect on the kidney in DN rats; and its mechanism of action might be related to down-regulated VEGF protein expression and up-regulated MMP-9 protein expression in kidney tissues.

Diabetic nephropathy (DN) is one of the major complications of diabetic microangiopathy (Onalan, 2019), and it has become a major cause of end-stage renal disease (Kumar *et al*., 2016). It is believed in academic circles that abnormalities in glucose and lipid metabolism (Herman-Edelstein *et al*., 2014), oxidative stress (Kashihara *et al*., 2010), and increased stimulation of advanced glycation end products (Thomas *et al*., 2005) are the main factors for the pathogenesis of DN, since these factors can stimulate renal cell proliferation and fibrosis, which is closely related to the abnormal expression of extracellular matrix (ECM). The progressive deposition and accumulation of ECM are the pathological basis for early DN lesions. Vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP-) 9 play a very important part in ECM deposition. VEGF is involved in the accumulation of mesangial ECM (Lee *et al*., 2012). During the accumulation of ECM, MMP-9, having exactly the opposite function to VEGF, plays a negative regulatory role and can promote the degradation of ECM (Gao *et al*., 2007).

It has been confirmed (Cohen *et al*., 2005; Sakamaki *et al*., 2010) that MMP-9 and VEGF are a pair of related factors, both of which are involved in occurrence of related renal diseases such as glomerulosclerosis and membranous glomerulonephritis. For diabetes, high glucose stimulation induces abnormally high expression of a variety of factors including VEGF, and promotes the up-regulated expression of various component genes of the mesangial matrix in the kidney, eventually leading to increased ECM synthesis and down-regulated MMP-9 expression (Tripathi *et al*., 2017). Therefore, the development of diabetic renal protection drugs with VEGF and MMP-9 as targets has a positive significance in delaying DN.

Tetrahydroxystilbene glucoside (TSG) is a water-soluble active ingredient extracted from polygonum multiflorum (Ning *et al*., 2018). At present, there have been few reports on the effects of TSG on VEGF and MMP-9 protein expression in renal tissues in rats with DN.

The purpose of this study was to investigate the intervention effects of TSG in DN rats and analyze its action on VEGF and MMP-9 protein expression in kidney tissues.

Materials and methods

Thirty-two healthy SD male rats weighing 185-220 g were selected and kept in a ventilated clean cabinet. After one week of adaptive feeding, they were randomly divided into the diabetic model group (n=22) and the normal control group (n=10).

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Rats in the model group were given high-fat diet, while rats in the control group were given ordinary diet for 4 weeks. After that, rats in the model group were injected intraperitoneally with 30 mg/kg streptozotocin, and rats in the control group with an equal volume of citric acid buffer solution. After 72 h, blood glucose was measured from the tail vein of the model rats, and the blood glucose ≥16.7 mmol/L for 2 consecutive times was defined as a sign of successful diabetes modeling.

After the diabetes models were successfully established, the rats were fed with high-fat diet for another 6 weeks. Blood glucose, urine glucose, urine volume, and urine microalbumin were monitored weekly. Blood glucose ≥16.7 mmol/L, urine glucose qualitative analysis >+++, urine volume >50% than that before the modeling, the urine microalbumin >50% than that before the modeling, and occurrence of early DN pathological changes in the kidney were regarded as signs of successful replication of the DN models (2 rats were excluded due to the failure of modeling).

Twenty successfully modeled rats were randomly divided into the model group and the TSG group, with 10 rats in each group. One week after successful modeling, the TSG group was given TSG intraperitoneally, at a dose of 20 mg/kg once daily. The rats in the control group and the model group were intraperitoneally injected with the same amount of citric acid buffer once a day. All animals were continuously administered for 12 weeks. During the administration period, rats in each group were fed with normal diet, with free access to food and routine drank water.

On the 12th week of administration, the 24 h urine sample was collected in a metabolic cage and sent for determination of 24 h urine protein.

At the end of the 12th week, blood was taken from the femoral artery before the rats were sacrificed. Serum was separated to determine serum creatinine (Scr), blood urea nitrogen (BUN), blood uric acid (UA), blood total protein (TP), blood albumin (ALB), blood alanine aminotransferase (ALT) and blood aspartate aminotransferase (AST).

Determination of VEGF and MMP-9 protein expression via Western blot. At the end of the 12th week of administration, the rats were sacrificed, and the kidneys were taken. The kidney tissue was cut into small pieces, added with RIPA lysate, and homogenized with a homogenizer until it was fully lysed. The lysed sample was centrifuged and the supernatant was taken to measure the protein concentration with bicinchoninic acid method. The 30 μg sample was loaded to perform 10% SDS-PAGE electrophoresis, and the protein was transferred to a piezoelectric polymer polyvinylidene fluoride membrane, blocked with 5% skim milk powder for 1 h, incubated at 4°C overnight with VEGF, MMP-9, β-actin primary antibodies, and incubated at room temperature for 1 h with biotin-labeled goat anti-mouse IgG secondary antibody for electro-chemiluminescence color development and exposure. Banding analysis was performed with a gel imaging analyzer. The average absorbance of the bands was detected, and the absorbance ratio of the target protein to the internal reference β-actin was measured in each well.

Data were analyzed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). One way analysis of variance (ANOVA) was used to determine the significance of each parameter among the groups, while LSD-t test was used for further comparison between every two groups. If the probability value (p) was less than 0.05, it was considered significant.

Results and discussion

After 12 weeks of administration, 24 h urine protein was higher in the normal control group than that in the model group (P <0.05, Fig. 1), and it was lower in the TSG group than that in the model group (P <0.05, Fig. 1). This indicates that TSG can reduce proteinuria and exert a certain effect in treating DN. This conclusion is basically consistent with the results reported in previous studies (Chen *et al*., 2016).

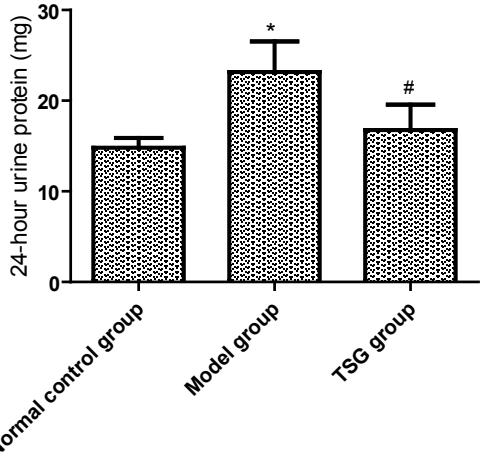

Fig. 1. Effect of TSG on the 24 h urine protein in DN rats. *Significant difference $p < 0.05$, compared with normal control group. #Significant difference $p < 0.05$, compared with model group. Data are expressed as mean ± SD (n=10).

Blood Scr, BUN, and UA were higher in the model group than those in the normal control group (P <0.05, Table I). After 12 weeks of administration, blood Scr, BUN, and UA in the TSG group were lower than those in the model group (P <0.05, Table I). This shows that

TSG can significantly reduce the production of blood creatinine, urea nitrogen and blood uric acid in rats with DN. This conclusion is basically consistent with the results of previous studies (Li *et al*., 2010) that TSG can significantly reduce blood urea nitrogen and creatinine in DN rats.

Table I. Comparison of serum biochemical indexes of rats in each group (Mean±SD,n=10).

Parameter	Normal control group	Model group	TSG group
Scr(μmol/L)	40.76±6.33	53.45±8.12*	41.56±7.39#
BUN(mmol/L)	4.02±0.95	18.39±5.07*	5.24±1.15#
UA(μmol/L)	117.53±9.16	201.68±13.21*	121.63±15.44#
TP(g/L)	70.16±5.25	51.05±3.94*	62.58±7.81#
ALB(g/L)	30.34±2.01	25.76±3.18*	28.04±2.55#
ALT(U/L)	50.62±6.37	118.43±9.02*	60.95±8.23#
AST(U/L)	175.77±14.18	346.91±18.33*	223.27±15.46#

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; BUN, blood urea nitrogen; Scr, serum creatinine; TP, total protein; TSG, tetrahydroxystilbene glucoside;UA, uric acid.
*Significant difference $p < 0.05$, compared with normal control group.
#Significant difference $p < 0.05$, compared with model group.

Blood TP and ALB were lower in the model group than those in the normal control group (P <0.05, Table I); and after 12 weeks of administration, both were higher in the TSG group than those in the model group (P <0.05, Table I). Blood ALT and AST were higher in the model group than those in the normal control group (P <0.05, Table I); and after 12 weeks of administration, both were lower in the TSG group than those in the model group (P <0.05, Table I). This indicates that TSG can reduce kidney damage and protect kidney function in DN rats.

Compared with that in the normal control group, VEGF protein expression in the model group significantly increased (P <0.05); and compared with that in the model group, VEGF protein expression in the TSG group significantly reduced (P <0.05, Fig. 2A and 2B).

Compared with that in the normal group, MMP-9 protein expression in the model group significantly reduced (P <0.05); and compared with that in the model group, MMP-9 protein expression significantly increased in the TSG group (P <0.05, Fig. 2A and 2B).

However, the pathogenesis of DN is more complicated. The mechanism by which TSG protects DN may also involve multiple aspects. It was found in this study that TSG could reduce proteinuria levels, slow down the progression of DN, and have a certain protective effect on the kidney in DN rats; and its mechanism of action might be related to down-regulated VEGF protein expression and up-regulated MMP-9 protein expression in kidney tissues.

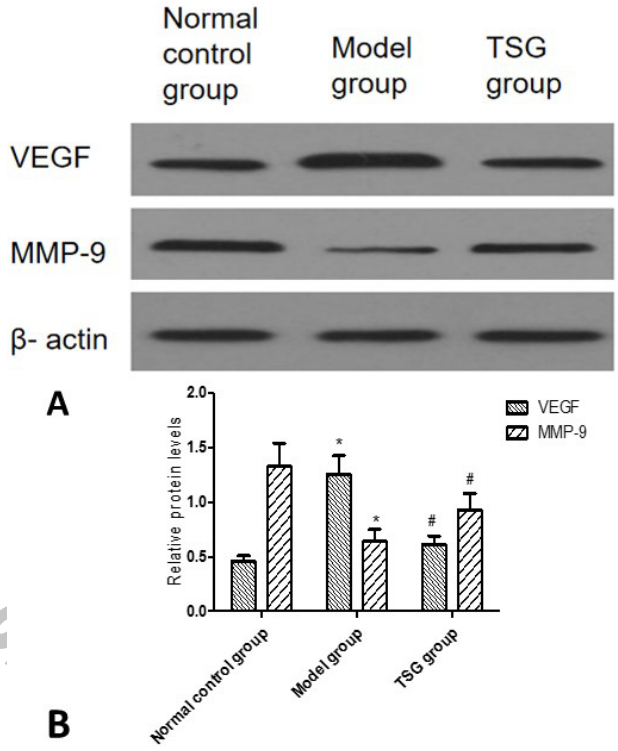

Fig. 2. Comparison of the expression of VEGF (Vascular endothelial growth factor) and MMP-9 (matrix metalloproteinase 9) protein in renal tissue. Results of Western blotting (A). Western blotting analysis(B).*Significant difference $p < 0.05$, compared with normal control group. #Significant difference $p < 0.05$, compared with model group. Data are expressed as mean ± SD (n=10).

The dose of TSG (20 mg/kg) used in this study is the high dose in reference to (Yuan *et al*., 2016). The effects of other doses of TSG on VEGF and MMP-9 protein expression in the kidney tissue of rats with DN still require further study.

Conclusion

The present study results showed that TSG could reduce proteinuria levels, slow down the progression of DN, and have a certain protective effect on the kidney in DN rats; and its mechanism of action might be related to down-regulated VEGF protein expression and up-regulated MMP-9 protein expression in kidney tissues.

Statement of conflict of interest

The authors have declared no conflict of interest.

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