Original Paper

Different Doses of *Tripterygium* Glycosides in the Treatment of Diabetic Nephropathy: Effects on Blood Lipids

Wei Wang

Department of Endocrinology, Tianjin First Center Hospital, Tianjin, China

Key Words

Diabetic nephropathy • *Tripterygium* glycosides (TG) • Proteinuria • Blood lipid

Abstract

Background/Aims: The aim of this study was to investigate the therapeutic effect of different doses of *tripterygium* glycosides (TG) in the treatment of diabetic nephropathy (DN). The effect of TG on blood lipids and safety were also evaluated. **Methods:** Sixty patients with type 2 DN were randomly divided into three groups ($n=20$ per group): low-dose (30 mg/day TG), double-dose (60 mg/day TG) and control (placebo) groups, all three times daily for 6 months. The levels of triglycerides, total cholesterol, urinary protein, plasma albumin and serum tumour necrosis factor (TNF)- α were compared between the three groups. **Results:** After treatment, urinary protein and TNF- α level significantly decreased in patients in the treatment groups, compared with the control group. This decrease was significantly larger in the double-dose group than in the low-dose group. However, there was no significant decrease in triglycerides and total cholesterol in the two treatment groups. Furthermore, plasma albumin was lower in the treatment groups than in the control group. **Conclusion:** The double dose of TG has improved clinical efficacy, compared with the low dose, and the same safety profile.

© 2018 The Author(s)
Published by S. Karger AG, Basel

Introduction

Diabetic nephropathy (DN) is the most common microvascular complication of diabetes mellitus. At present, the pathogenesis of DN has not been fully elucidated. Most researchers have considered that disorders of glucose and lipid metabolism, abnormal renal haemodynamic parameters and renin-angiotensin system dysfunction play key roles in the occurrence and development of DN [1]. Some have also considered that cytokines, inflammatory mechanisms [2], oxidative stress and podocyte injury [3] are important in the pathogenesis of DN. In recent years, the combination of Western and Chinese patent medicine

has become popular in the treatment of DN, and satisfactory therapeutic effects have been achieved. *Tripterygium wilfordii* can stabilise the skeleton structure of podocytes and even reverse existing podocyte injury, restore podocyte structure and the expression of related molecules, and accordingly reduce urinary protein level [4]. After DN rats were treated with *Tripterygium wilfordii* for 12 weeks, the foot processes of the podocytes fused, the slit diaphragm disappeared, and the number of fissure holes decreased. These improvements were significantly larger with an increase in the dosage of *Tripterygium wilfordii* [5]. The effect of *Tripterygium wilfordii* on cytokines has been widely confirmed; some studies indicated that *Tripterygium wilfordii* suppressed production of cytokines, including tumour necrosis factor (TNF)- α , interleukin (IL)-2, interferon (IFN)- γ , IL-6 and IL-8 in serum [6, 7]. However, its clinical application is limited by side effects. In the present study, the therapeutic effect of different doses of *tripterygium* glycosides (TG; a component of *Tripterygium wilfordii*) in the treatment of DN and its effect on blood lipid level were investigated, and the safety of different doses was evaluated.

Materials and Methods

Study design

Patients who were admitted to the Department of Endocrinology of our hospital from January to December 2014 were enrolled in this study. These patients comprised 30 men and 30 women. Patients were included if they met the following criteria: (1) World Health Organization criteria for the diagnosis of type 2 diabetes mellitus [8] and the Mogensen criteria for the diagnosis and staging of DN [9]; (2) renal biopsy in the past 6 months and the pathological diagnosis of DN, or no renal biopsy but the clinical diagnosis of DN; (3) 24-h urinary protein >1.0 g, glomerular filtration rate >60 ml/min/1.73 m² and serum creatinine (SCr) <133 mol/L; (4) no other hepatorenal disease and a leukocyte count $>3.5 \times 10^9$ /L; (5) stable blood glucose levels and glycated haemoglobin (HbA1C) $<7\%$; (6) blood pressure (BP) stabilised by conventional doses of angiotensin-converting enzyme inhibitor or arylsulfatase B (BP $<140/80$ mmHg); and (7) good compliance, approval to participate by the ethics committee of the hospital and written informed consent. Patients were excluded if they met the following criteria: (1) diagnosis of type 1 diabetes mellitus; (2) no diagnosis of DN; (3) severe hypertension that was difficult to control; (4) active hepatitis; (5) infections, such as pulmonary infection, cellulitis of the skin and soft tissue and recurrent upper respiratory tract infection, in the past month; and (6) severe cardiovascular or cerebrovascular events, including angina pectoris, myocardial infarction, heart failure, cerebral infarction or cerebral haemorrhage, in the past 6 months. TG was obtained from Meitong Pharmaceutical Co. Ltd. (Jiangsu, China), and the content of TG of each tablet was 10 mg. The drug is a basic medicine in the national medical insurance catalogue, and was purchased by our hospital. The extraction method and production process of TG were both subject to strict quality control, and the main components were standardised [10]. In addition, TG is prepared into granules after dynamic circulatory extraction and concentration by evaporating and spray drying and is also monitored for the absence of contaminants (heavy metals, pesticides, hormones and mycotoxins) prior to formulation. The quality of TG was examined by fingerprint analysis using high-performance liquid chromatography in Jiangsu Province Academy of Chinese Medicine (Nanjing, China). The known bioactive components including triptolide (C₂₀H₂₄O₆; CAS, 38748-32-2), triptonide (C₂₀H₂₂O₆; CAS, 38647-11-9), wilforgine (C₄₁H₄₇NO₁₉; CAS, 37239-47-7), wilforine (C₄₃H₄₉O₁₈; CAS, 11088-09-8), tripterine (C₂₉H₃₈O₄; CAS, 34157-83-0) and wilforlide A (C₃₀H₄₆O₃; CAS, 84104-71-2) in five batches exhibited high stability [11]. In order to avoid the impact of differences in drug quality, the same batch number was specifically chosen. According to the manufacturer's instructions, the drug should be taken orally at 1–1.5 mg/kg/day for the treatment of nephrotic syndrome. Therefore, the base dose was set at 30 mg. The drug has frequently been used in the clinical treatment of DN, but has not been introduced into treatment guidelines. This study was conducted with approval from the local ethics committee.

Treatment groups

Patients were randomly divided into three groups (20 patients per group), according to the random number table: (1) the control group comprised 9 men and 11 women aged 49–65 years (disease duration: 7–15 years); (2) the low-dose group comprised 10 men and 10 women aged 50–66 years, treated with TG (disease duration: 8–16 years); and (3) the double-dose group comprised 11 men and 9 women aged 51–64 years, treated with a double dose of TG (disease duration: 9–16 years). Differences in sex, age, disease duration, BP, urinary protein content and SCr among these three groups were not statistically significant.

Therapeutic methods

Patients in the three groups routinely received hypoglycaemic treatment, and the antidiabetic measures were not limited (including oral hypoglycaemic agents or insulin injection). Fasting and 2-h postprandial blood glucose and HbA1C were monitored. The blood glucose control range was: fasting blood glucose <7 mmol/L, 2-h postprandial blood glucose <10.0 mmol/L and HbA1c <7%. All patients underwent BP management, and were given an appropriate low protein diet (0.6–0.8 g/kg/day) and symptomatic support therapy. On this basis, patients in the low-dose group were treated with 30 mg/day TG three times daily (*tid*), while patients in the double-dose group were treated with 60 mg/day TG *tid*. The duration of treatment was 6 months.

Outcomes

Changes in urinary protein, serum albumin, renal function, blood lipids and TNF- α level in the three groups after treatment were recorded and analysed. Furthermore, adverse reactions during the course of treatment were monitored.

Statistical analysis

Data were analysed using SPSS 16.0 statistical software. All data were expressed as mean $\pm$ standard deviation. Multi-group comparisons were conducted using univariate analysis of variance, and comparisons between two groups were conducted using *q*-test. $P < 0.05$ was considered statistically significant.

Results

Comparisons before treatment among the three groups (Table 1)

The differences in baseline levels of general indices before treatment among the three groups were not statistically significant ($P > 0.05$).

Comparisons after treatment among the three groups (Table 2)

After 6 months of treatment, differences in total cholesterol, triglycerides and SCr among the three groups were not statistically significant, whereas differences in urinary protein content, plasma albumin and TNF- α level were statistically significant ($P < 0.05$). Decreases in urinary protein content and TNF- α level were significantly larger in the double-dose group than in the low-dose group ($P < 0.05$). Plasma albumin decreased in the two TG treatment groups to a greater extent than in the control group ($P < 0.05$).

Adverse reactions

No adverse reactions occurred in the control or low-dose groups. In the double-dose group, one patient had an elevated level of alanine aminotransferase, but this did not exceed two times the baseline level. This immediately returned to the baseline level after symptomatic treatment and did not affect the course of treatment.

Table 1. Comparisons of general data before treatment among the three groups. Note: TNF, tumour necrosis factor

Variable	Double dose group	Low dose group	Control group	F value	P value
Gender (male/female)	9/11	10/10	11/9		
Age (years)	58±3.8	59±4.2	58±5.9	0.30	0.74
Disease course (years)	16±2.3	15±3.4	14±3.5	2.06	0.14
Systolic pressure (mmHg)	132±12.3	133±11.5	134±12.9	0.13	0.88
Diastolic pressure (mmHg)	81.6±10.7	83.2±9.8	85.6±8.5	0.86	0.43
HbA1c (%)	6.22±2.23	6.32±2.15	6.42±1.42	0.05	0.95
GLU (mmol/L)	5.98±1.25	6.02±1.31	6.13±1.23	0.08	0.93
CHOL (mmol/L)	5.68±1.13	5.79±1.56	5.97±1.23	0.25	0.78
TG (mmol/L)	1.73±0.53	1.78±0.68	1.69±0.45	0.13	0.88
ALB (g/L)	35.35±5.67	36.32±4.59	35.64±3.79	0.22	0.80
SCr (μmol/L)	108.69±24.68	109.65±30.65	108.56±26.58	0.01	0.99
Urine protein (g/24h)	2.34±0.68	2.45±0.75	2.58±0.98	0.44	0.65
TNF-α (ng/L)	219.03±12.35	218.36±15.46	219.20±13.45	0.02	0.98

Table 2. Comparisons of indicators after treatment among the three groups. Note: TNF, tumour necrosis factor

Variable	Double dose group	Low dose group	Control group	F value	P value
CHOL (mmol/L)	5.53±1.78	5.61±1.13	5.86±1.45	0.27	0.76
TG (mmol/L)	1.71±0.73	1.72±0.87	1.67±0.68	0.02	0.98
ALB (g/L)	31.32±5.63	32.65±3.68	34.65±4.53	2.56	0.09
SCr (μmol/L)	110.45±26.68	109.75±24.58	107.68±23.88	0.07	0.94
Urine protein (g/24h)	1.08±0.76	1.72±0.78	2.13±0.67	10.28	0.00
TNF-α (ng/L)	103.25±15.74	156.23±12.68	210.56±14.58	278.12	0.00

Discussion

At present, the pathogenesis of DN has not been fully elucidated. Most researchers have considered that disorders of glucose and lipid metabolism, renal haemodynamic indices and renin-angiotensin system dysfunction play key roles in the occurrence and development of DN [1]. Others have considered that cytokines, inflammatory mechanisms [2], oxidative stress and podocyte injury [3] are important in the pathogenesis of DN. DN is mainly characterised by glomerulosclerosis, which leads to massive proteinuria, abnormally high BP and secondary renal function impairment. Proteinuria is the primary clinical manifestation of DN, and is also an independent risk factor for the progression of DN [12], which can further aggravate glomerulosclerosis and renal sclerosis, and seriously affect the clinical prognosis.

Tripterygium wilfordii, a member of the *Celastraceae* family, is also known as Litouciteng and Huangtenggen, and its name in Chinese loosely translates as “intestine-breaking grass”. It has a sour and bitter taste, and its drug property is neutral. Its major effects include reducing inflammation, detoxification and acting as an insecticide. TG is an effective component extracted from the root core of *Tripterygium wilfordii* with water and chloroform, and separated by column chromatography. It is a mixture of systematic liposoluble components.

In this study, after 6 months of TG treatment, the levels of total cholesterol and triglycerides slightly decreased in the two treatment groups, but the differences before and after treatment were not statistically significant, while urinary protein and TNF-α levels significantly decreased in the two treatment groups, and the decrease was significantly larger in the double-dose group. Furthermore, the level of plasma albumin decreased somewhat in the two treatment groups, but the difference between the double-dose and low-dose groups was not statistically significant. The reason may be the effect of TG on the liver. However, there was no further reduction in plasma albumin due to the doubling of the dose.

The most important characteristic of TG is a powerful immunosuppressive role; it does not cause serious damage to the normal immune system which would cause severe infection or tumours. Its immunosuppressive effects mainly manifest as changes in inflammatory factors and cytokines, and it also promotes repair of podocytes in DN patients. An important link in the occurrence and development of DN is the injury of podocytes. The podocyte is one of the main cell types that maintain the normal structure and function of glomerular filtration membranes [13]. In DN patients, fusion of podocytes, disappearance of the fissure membrane and abscission of podocytes can be observed. Deletion of the expression of cell fissure membrane protein nephrin and decrease in the expression of podocyte-related molecule WT1 can be detected by immunohistochemistry. *Tripterygium wilfordii* not only stabilises the skeleton structure of podocytes, but also reverses existing podocyte injury, restores the skeleton structure and the expression of related podocyte molecules and accordingly reduces urinary protein [4]. Zheng et al. [5] revealed that after treatment of DN rats with *Tripterygium wilfordii* for 12 weeks, foot process fusion, slit diaphragm disappearance and a reduced number of fissure holes in podocytes were observed, and these changes became clearer with an increase in the dose of *Tripterygium wilfordii*.

The effects of TG on inflammatory factors and oxidative stress have been reported in many studies. In the process of early DN, microinflammation and activation of p38 mitogen-activated protein kinase (MAPK) and canonical nuclear factor- κ B (NF- κ B) signalling pathways are important mechanisms through which hyperglycaemia contributes to glomerulosclerosis. Wu et al. [11] revealed that TG improved the general condition and biochemical parameters of rats, but did not decrease blood glucose; TG attenuated glomerulosclerosis and suppressed glomerular microinflammation including the infiltration of ED1⁺ cells in glomeruli and the overexpression of TNF- α , IL-1 β and TGF- β 1 in the kidney. In addition, TG inhibited the overexpression of key signalling molecules of p38 MAPK and canonical NF- κ B pathways in the kidney including phosphorylated p38 MAPK, and phosphorylated inhibitor proteins I κ B and NF- κ B (p65). Furthermore, the effect of TG is dose dependent. Navarro et al. [14] found that the level of TNF- α in the urine of patients with type 2 diabetes mellitus significantly increased, compared with healthy controls. In addition, with the increase in urinary protein excretion rate, urinary TNF- α level also increased. This suggests that TNF- α plays an important role in the pathogenesis and progression of DN. Wang et al. [15] also revealed that the level of C-reactive protein (CRP) in serum of DN patients clearly increased. CRP leads to oxidative stress in the body through an inflammatory response, and directly causes damage to glomerular endothelial cells. In addition, it enhances the adhesion and infiltration of monocytes to renal vascular endothelial cells. The present results also confirmed that TG had a positive effect in reducing TNF- α levels, and this effect was increased with a higher dose. In addition, the specific chemotactic factor monocyte chemoattractant protein 1 (MCP-1) plays an important role in the chemotaxis and activation of renal inflammatory cells [16, 17]. The exacerbation of tubulointerstitial damage induced by MCP-1 was correlated to fibrosis and tubule atrophy of the renal interstitium [18]. Zhang et al. [19] demonstrated in a rat model that the expression of MCP-1 in renal tissue significantly increased in the DN group. However, this expression significantly decreased after TG treatment. Meanwhile, through measurement of the oxidative stress markers lipid peroxidation product malondialdehyde and superoxide dismutase (SOD), which are enzymes that eliminate superoxide anion free radicals, the authors also revealed that TG has marked advantages in improving oxidative stress, compared with irbesartan. The manifestations are effective reduction of the expression of malondialdehyde in renal tissues of DN rats and improved SOD activity. In particular, the effect of double-dose therapy was significantly larger. TG also reduced albuminuria, protected renal function and increased blood albumin.

The effect of *Tripterygium wilfordii* on cytokines has been extensively confirmed. Activin is a recently discovered member of the transforming growth factor (TGF)- β superfamily, and TGF- β , which is well recognised as an important factor for fibrosis, is a marker of transdifferentiation of tubular epithelial cells into fibroblasts. Activin can promote the

differentiation of renal tubular epithelial cells, promote the proliferation of interstitial cells, and has a role in fibrosis of the renal interstitium [20]. It was shown that there was almost no expression of activin in the kidneys of healthy adults, and the expression of activin in renal tissue in patients with ischaemia-reperfusion injury was significantly increased [7]. Zhao et al. [21] revealed that TG inhibited Act, and accordingly blocked the transdifferentiation of the renal tubulointerstitium and delayed fibrosis of the kidneys, thereby protecting the kidneys.

In this study, after rats were treated with different doses of TG (4.5, 9.0 and 18.0 mg/kg), there were no significant changes in the low-dose and control groups. However, in the medium and high-dose groups, the clearance rate of 24-h microalbuminuria, blood urea nitrogen (BUN) and endogenous creatinine significantly decreased and glomerular hypertrophy, mesangial cell proliferation and glomerular deformation were clearly reduced, compared with the control group. These effects were positively correlated with increasing dosage. In the present study, creatinine levels did not decrease in the double-dose group, possibly because the renal function of the subjects enrolled in the study was in the normal range.

This study has some limitations. Due to the small number of patients included in this study, observation of the level of urinary protein was not sufficient to fully understand the outcome of ND. Moreover, follow-up of patients for longer periods would provide more definitive results.

Conclusion

TG can delay the progression of DN through several approaches, involving inflammatory factors and podocyte repair. Furthermore, TG has the advantages of high efficiency and safety, and low cost, with strong clinical evidence for use in the treatment of DN.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

- 1 Lizicarova D, Krahulec B, Hirnerova E, Gaspar L, Celecova Z: Risk factors in diabetic nephropathy progression at present. *Bratisl Lek Listy* 2014;115:517-521.
- 2 Lim AK, Tesch GH: Inflammation in diabetic nephropathy. *Mediators Inflamm* 2012;2012:146154.
- 3 Gnudi L: Cellular and molecular mechanisms of diabetic glomerulopathy. *Nephrol Dial Transplant* 2012;27:2642-2649.
- 4 Zheng CX, Chen ZH, Zeng CH, Qin WS, Li LS, Liu ZH: Triptolide protects podocytes from puromycin aminonucleoside induced injury *in vivo* and *in vitro*. *Kidney Int* 2008;74:596-612.
- 5 Zheng Y, Hao L, Pan MS, Ding N: Effect of tripterygium wilfordii polyglycoside on the podocytes of diabetic nephropathy rats. *Chin J Nephrol* 2011;4:288-292.
- 6 Jingjing Wang, Yi Chu, Xiaoying Zhou: Inhibitory effect of Tripterygium wilfordii polyglucoside on dipeptidyl peptidase I *in vivo* and *in vitro*. *Biomedicine & Pharmacotherapy* 2017;96:466-470.
- 7 Maeshima A, Zhang YQ, Nojima Y, Naruse T, Kojima I: Involvement of the activin-follistatin system in tubular regeneration after renal ischemia in rats. *J Am Soc Nephrol* 2001;12:1685-1695.
- 8 Heilig CW, Deb DK, Abdul A, Riaz H, James LR, Salameh J, Nahman NS Jr: Glut1 regulation of the pro-sclerotic mediators of diabetic nephropathy. *Am J Nephrol* 2013;38:39-49.

- 9 Krist J, Wieder K, Klötting N, Oberbach A, Kralisch S, Wiesner T, Schön MR, Gärtner D, Dietrich A, Shang E, Lohmann T, Dreßler M, Fasshauer M, Stumvoll M, Blüher M: Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. *Obes Facts* 2013;6:57-69.
- 10 Li K, Wang S: Fingerprint chromatogram analysis of extracts from the leaves of *Tripterygium wilfordii* Hook. f. by high performance liquid chromatography. *J Sep Sci* 2005;28:653-657.
- 11 Wu W, Yang JJ, Yang HM, Huang MM, Fang QJ, Shi G, Mao ZM, Han WB, Shen SM, Wan YG: Multi-glycoside of *Tripterygium wilfordii* Hook. f. attenuates glomerulosclerosis in a rat model of diabetic nephropathy by exerting anti-microinflammatory effects without affecting hyperglycemia. *Int J Mol Med* 2017;40:721-730.
- 12 Kowalski A, Krikorian A, Lerma EV: Diabetic nephropathy for the primary care provider: new understandings on early detection and treatment. *Ochsner J* 2014;14:369-379.
- 13 Shankland SJ: The podocyte's response to injury: role in proteinuria and glomerulosclerosis. *Kidney Int* 2006;69:2131-2147.
- 14 Navarro JF, Mora C, Muros M, García J: Urinary tumour necrosis factor- α excretion independently correlates with clinical markers of glomerular and tubulointerstitial injury in type 2 diabetic patients. *Nephrol Dial Transplant* 2006;21:3428-3434.
- 15 Wang HY: Correlation between proteinuria and CRP level in patients with Diabetic Nephropathy. *China Prac Med* 2011;6:41-43.
- 16 Mora C, Navarro JF: The role of inflammation as pathogenic factor in the development of renal disease in diabetes. *Curt Diab Rep* 2005;5:399-401.
- 17 Elmmarakby AA, Sullivan JC: Relationship between oxidative stress and inflammatory cytokines in diabetic nephropathy. *Cardiovasc Ther* 2012;30:49-59.
- 18 El Mesallamy HO, Ahmed HH, Bassyouni AA, Ahmed AS: Clinical significance of inflammatory and fibrogenic cytokines in diabetic nephropathy. *Clin Biochem* 2012;45:646-650.
- 19 Zhang JQ, Zhang Y, Yin XL, Yang P, Zhang HF, Guo YL, Chen WD: Effects of Tripterygium Glycosides on Expressions of MCP-1 and CTGF in Rats with Early Diabetic Nephropathy. *Trop J Pharm Res* 2016;15:55-64.
- 20 Liu GH, Wang LH: Expression of activin A in experimental renal fibrosis. *CJITWN* 2008;9:816-817.
- 21 Zhao CG, Yu WM, Ren XJ, Zhang W, Li H: Effects of tripterygium wilfordii polyglucoside on expression of Activin A and influence of transdifferentiation in renal tubulointerstitium of rats with diabetic nephropathy. *Natl Med J China* 2014;94:1427-1432.