TRIPTERYGIUM WILFORDII POLYGLYCOSIDES INHIBIT THE TGF-BETA 1/NF-KAPPA B SIGNALLING PATHWAY AND IMPROVE RENAL FIBROSIS IN DIABETIC NEPHROPATHY

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ABSTRACT

Objective: *The objective is to explore the mechanism of Tripterygium wilfordii polyglycosides (TWP) in improving fibrosis in diabetic nephropathy by inhibiting the TGF-beta 1/NF-kappa B signalling pathway.*

Methods: *Fifty clean SD rats were randomly selected, and 10 of them were in the normal control group. The remaining 40 rats were injected intravenously with streptozotocin to establish the model of type 2 diabetes mellitus. After successful modelling, the rats were randomly divided into a model group and low-, medium-, and high-dose TWP groups (1, 3, and 6 mg/kg/d), with 10 rats in each group. The normal control group and model group were given the same amount of distilled water by gavage. The TWP groups were given different doses of TWP by gavage, and the rats were sacrificed after 8 weeks of gavage. The changes in renal function, urinary microalbumin (UMA), and blood lipids in rats were detected using blood samples. The levels of serum TGF-beta 1 and NF-kappa Bp65 were detected using ELISA, and the levels of TGF-beta 1 and NF-kappa Bp65 were detected using Western blotting.*

Results: *Compared with the control group, the levels of triglycerides and total cholesterol in the model groups were significantly higher (P<0.05), whereas the levels of high-density lipoprotein cholesterol were not significantly different (P>0.05). The levels of UMA in the model group were significantly higher than those in the control group (P<0.05), and the levels of UMA in the TWP rats in each dose group were significantly lower than those in the model group (P<0.05). The levels of UMA in the medium- and high-dose TWP groups were significantly lower than those in the low-dose group (P<0.05). The serum levels of TGF-beta 1 and NF-kappa Bp65 in the model group were significantly higher than those in the control group (P<0.05). After different doses of TWP treatment, the serum levels of TGF-beta 1 and NF-kappa Bp65 in the middle- and high-dose groups were significantly lower than those in the model group (P<0.05). Compared with the control group, the levels of TGF-beta 1 and NF-kappa Bp65 in the kidney tissue of the model group were significantly higher than those in the control group (P<0.05). Compared with the model group, the expression levels of TGF-beta 1 and NF-kappa Bp65 in the kidney tissue of rats in each dose group were significantly lower (P<0.05), and the expression levels of TGF-beta 1 and NF-kappa Bp65 in the middle- and high-dose TWP groups were significantly lower than those in low-dose group (P<0.05).*

Conclusion: *The TGF-beta 1/NF-kappa B signalling pathway mediates the occurrence and development of renal fibrosis. Tripterygium wilfordii polyglycosides can improve diabetic renal fibrosis by inhibiting the TGF-beta 1/NF-kappa B signalling pathway in a dose-dependent manner.*

Keywords: *Tripterygium wilfordii polyglycosides, TGF-β1/NF-ϰB signalling pathway, diabetic nephropathy, renal fibrosis.*

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Introduction

Diabetic nephropathy (DN) is the most serious and harmful chronic complication of diabetes mellitus. It mainly affects the glomerulus, leading to an increase in the glomerular filtration rate and protein filtration rate. Its clinical manifestations are mainly renal structural damage and renal dysfunction. It has become the first cause of end-stage renal disease and an important factor leading to death in the later stages of diabetic development[1-2]. Studies have found that tubulointerstitial fibrosis is the last common pathway of most chronic types of progressive nephropathy, including DN. Moreover, TGF-β1 is a fibrogenic growth factor, which can inhibit cell mitosis and promote the production of the extracellular matrix, such as mesangial cells and glomerular epithelial cells. The production of the extracellular matrix can also

act on the mesangium and tubules, leading to further sclerosis of glomeruli, promoting the hypertrophy of kidney tissue, and aggravating the process of failure[3]. In addition, mesangial cells can synthesise and release a variety of cytokines to participate in glomerular inflammation. The NF-κB inflammatory signalling pathway is an important transcriptional regulator in cells. It plays an important role in cell apoptosis and cell cycle regulation. It has been confirmed that inflammatory factors can induce the transformation of TGF-β1 into an activated state in alveolar epithelial cells and participate in a variety of biological effects after activation. The activation form of NF-κBp65 is closely related to the occurrence and development of pulmonary fibrosis[4]. Some data show that Tripterygium wilfordii polyglycoside (TWP) has strong anti-inflammatory and immunoregulatory effects, inhibits the expression of many cytokines and adhesion factors, reduces the inflammatory damage of the glomerular mechanical barrier, and delays the development of DN[5]. However, the mechanism of TWP on renal fibrosis and extracellular matrix deposition in diabetic rats is unclear. In this study, streptozotocin was used to establish the DN rat model. The effect of TWP on the TGF-beta 1/NF-kappa B signalling pathway was analysed to further explore the role of TWP in DN and its mechanism.

Materials and methods

Laboratory Animals

Fifty clean male SD rats aged 4-8 weeks with a body mass of 190 g (+15) were purchased from Beijing Huafukang Science and Technology Co., Ltd. The tested animals were fed in separate cages (7-9 animals) at room temperature of 22°C to 25°C and humidity of 50% to 60% in an SPF grade animal room. The light and dark times were 12 hours, respectively, and the animals were fed freely with drinking water. The normal control group was fed a conventional diet, whereas the rest of the rats were fed a high-sugar and high-fat diet. The feed was disinfected using cobalt 60 irradiation.

Reagents and Instruments

The following were used in the study:

• Tripterygium wilfordii polyglycoside tablets (specification: 10 mg/tablet, batch number: 1203136, Zhejiang Deende Pharmaceutical Co., Ltd.);

• Streptozotocin (Sigma Company, United States);

• A rat enzyme-linked immunosorbent assay (ELISA) kit (Tianjin Haos Biotechnology Co., Ltd.);

• A protease inhibitor, bicinchoninic acid assay (BCA) protein concentration determination kit (Beijing Solarbio Company);

• A rat NF-kappa Bp65 anti-antibody (CST Company, United States);

• A beta-1 antibody (Abcam Company, UK); beta-actin antibody (Tianjin Sanjian Biotechnology Co., Ltd.);

• A concentrated immunohistochemical staining DAB kit (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.).

The following instruments were used in this study:

• A real-time fluorescence quantitative PCR instrument LightCycle (Switzerland Roche Company);

• A high-speed refrigerated centrifuge (German Herseus Company);

• A BA110S type electronic balance (Germany Sartorius Company);

• A vertical electrophoresis apparatus and electrical transfer device (BIO-RAD Company);

• An automatic gel imaging analysis system (American BD Company);

• An optical microscope and microphotography (Japan OLYMPUS Company).

Model Establishment

After 8 weeks of feeding, the rats were fed with fasting water for 12 hours. The blood from the medial canthal vein was taken to detect the levels of blood sugar, lipids, and fasting islet. After 3 days of stabilisation, the rats in the high-sugar and high-fat diet group were fed with fasting water for 12 hours. Then, rats were weighed. The rats were injected with 30 mg/kg of streptozotocin into the tail vein at one time to establish the model of type 2 diabetes mellitus (T2DM) rats. The normal control group was given 0.1 mol/L of citric acid-sodium citrate buffer. After successful modelling, the rats were randomly divided into a model group and low-, medium-, and high-dose TWP groups, with 10 rats in each group. The dosage of TWP was 1 mg/kg/d (low-dose group), 3 mg/kg/d (medium-dose group), and 6 mg/kg/d (high-dose group). The samples were sacrificed at 8 weeks. The control group and model group were given the same amount of distilled water. The drug dosage was adjusted according to the weight change of the rats, and the general situation of the rats was monitored.

Observation Indicators

After the rats were sacrificed, 2 ml of arterial blood was collected and placed in the anticoagulant tube of ethylenediaminetetraacetic acid

(EDTA). After centrifugation, the supernatant was taken. The renal function (serum creatinine [SCr] and blood urea nitrogen [BUN]) and blood lipids (total cholesterol [TC] and high-density lipoprotein (HDL) cholesterol) of the rats were detected using an automatic biochemical analyser. The changes in HDL cholesterol and triglycerides (TG) were also assessed. The serum levels of the TGF-beta 1 and NF-kappa Bp65 were measured using an ELISA kit. Urine microalbumin (UMA) levels over 24 hours in the urine of the rats were measured using Roche's analyser. The expression of TGF-beta 1 and NF-kappa Bp65 in the kidney tissue of the rats was detected using Western blotting.

Statistical Method

The SPSS (v. 19.0) software package was used to analyse all the research data. The standard deviation (SD) was used to express the measurement data. The T-test was used to compare the data between groups. One-way variance was used to compare the data among the groups, and $P < 0.05$ was used to determine the statistical significance of the difference.

Results

Changes in the Blood Lipid Levels in Rats of Each Group

At the end of 8 weeks, compared with the control group, the levels of TG and TC in the model group were significantly higher (P<0.05), whereas the levels of HDL cholesterol were not significantly different (P>0.05). The levels of blood lipids in the TWP dose groups were not significantly lower than those in the diabetes mellitus (DM) group (P>0.05). See Table 1.

Group	Cases	TG (mmol/L)	TC (mmol/L)	HDL-C (mmol/L)
Control	10	1.52±1.20	2.23±1.20	0.61±0.15
Model	10	4.45±1.87*	6.62±0.91*	0.52±0.11
Low-dose TWP	10	4.01±2.23*	6.65±2.13*	0.51±0.11
Medium-dose TWP	10	3.74±0.82*	6.42±2.23*	0.53±0.10
High-dose TWP	10	3.52±1.41*	6.23±2.00*	0.54±0.13

Table. 1: Comparison of the serum lipid levels in the rats of each group ($\bar{x}\pm SD$).

*Notes: *Representation compared with the control group (P<0.05). TWP: Tripterygium wilfordii polyglycosides TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol.*

Comparison of Renal Function and UMA Levels in Rats from Different Groups

There was no significant difference in BUN, SCr, and UMA levels among the groups before gavage (P>0.05). At the end of 8 weeks, there was no significant difference in BUN and SCr levels among the groups (P>0.05). The UMA levels in the model group were significantly higher than those in the control group (P<0.05). The UMA levels in the TWP rats in each dose group were significantly lower than those in the model group (P<0.05) and in the medium- and high-dose TWP groups. The UMA level was significantly lower than that in the low-dose group (P<0.05). See Table 2.

Group	BUN (mmol/L)		SCr (μmol/L)		UMA (μg/24h)	
	Time	8 W at the end of gavage	Time	8 W at the end of gavage	Time	8 W at the end of gavage
Control	8.41±1.12	10.92±2.01	36.61±2.62	32.84±4.40	41.23±11.20	45.71±14.52
Model	8.10±1.11	10.72±1.65	36.42±2.74	31.32±3.33	45.63±11.62	253.71±53.01*
Low-dose TWP	8.13±0.91	10.92±1.34	37.12±2.34	32.84±3.56	44.45±19.23	183.56±78.47*#
Medium-dose TWP	8.02±1.03	10.10±1.93	37.74±2.74	31.15±4.13	42.61±16.87	123.65±74.58*#△
High-dose TWP	8.24±1.23	10.26±1.25	37.34±2.74	31.56±3.84	48.85±13.20	119.03±52.14*#△

Table. 2: Comparison of renal function and UMA levels in rats from different groups ($\bar{x}\pm SD$).

*Note: *Representation compared with the control group (P<0.05); #Representation compared with the model group (P<0.05); △Representation compared with the low-dose TWP group (P<0.05). TWP: Tripterygium wilfordii polyglycosides; BUN: blood urea nitrogen; SCr: serum creatinine; UMA: urinary microalbumin; W: weeks.*

Changes in Serum Levels of TGF-β1 and NF-κBp65 in Rats

The serum levels of TGF-β1 and NF-κBp65 in the model group were significantly higher than those in the control group (P<0.05). After different doses of TWP treatment, the serum levels of TGF-β1 and NF-κBp65 in the middle- and high-dose groups were significantly lower than those in the model group (P<0.05). See Figure 1.

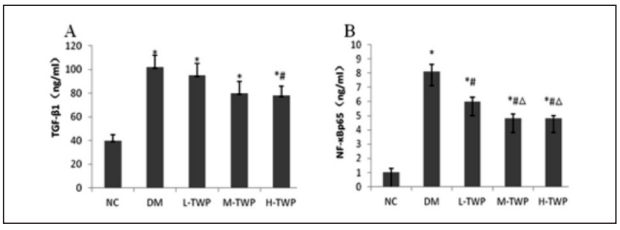

Figure 1: Changes of serum levels of TGF-β1 and NF-κBp65 in rats of each group.

*Note: *Representation compared with the control group (P<0.05); #Representation compared with the model group (P<0.05); △Representation compared with the low-dose TWP group (P<0.05). NC: normal control group; DM: diabetes mellitus, L-TWP: low-dose Tripterygium wilfordii polyglycosides; M-TWP: medium-dose Tripterygium wilfordii polyglycosides; H-TWP: high-dose Tripterygium wilfordii polyglycosides.*

Variations in TGF-β1 and NF-κBp65 Levels in Rat Kidneys

Compared with the control group, the levels of TGF-β1 and NF-κBp65 in the kidney tissue of the model group were significantly higher (P<0.05). Compared with the model group, the levels of TGF-β1 and NF-κBp65 in the kidney tissue of the rats in each dose group were significantly lower (P<0.05), and the levels of TGF-β1 and NF-κBp65 in the middle- and high-dose TWP groups were significantly lower than those in the low-dose group (P<0). See Figure 2.

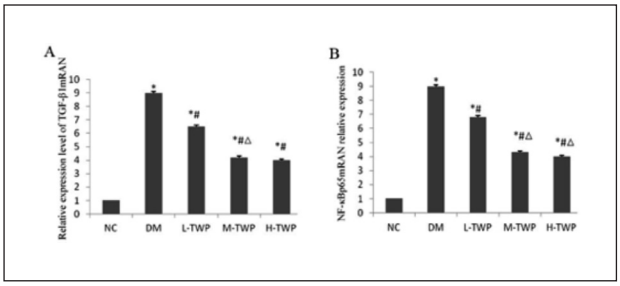

Figure 2: Changes in the TGF-β1 and NF-κBp65 levels in the kidney tissues of rats in each group.
*Note: *Representation compared with the control group (P<0.05); #Representation compared with the model group (P<0.05); △Representation compared with the low-dose TWP group (P<0.05).*

Western Blotting to Detect the Expression of TGF-β1 and NF-κBp65 in Rat Kidneys

The expression of TGF-β1 and NF-κBp65 in the kidney tissue of the model group rats was significantly higher than that of control group rats (P<0.05). The expression of TGF-β1 and NF-κBp65 in the kidney tissues of the TWP rats in each dose group was significantly lower than that of the model group rats (P<0.05). The expression of TGF-β1 and NF-κBp65 in the middle- and high-dose TWP groups was significantly lower than that in the low-dose group (P<0.05). See Figure 3.

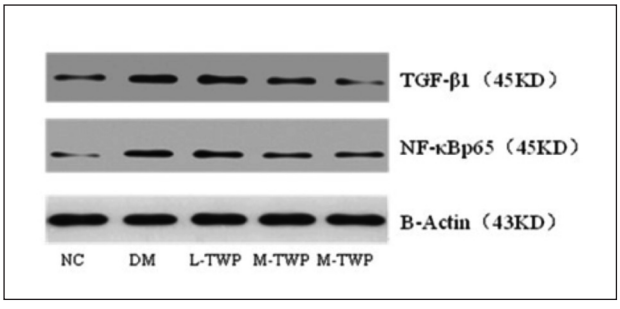

Figure 3: Expression of TGF-β1 and NF-κBp65 in rat kidneys.
Note: NC: normal control; DM: Diabetes mellitus; L-TWP: low-dose Tripterygium wilfordii polyglycosides; M-TWP: medium-dose Tripterygium wilfordii polyglycosides; H-TWP: high-dose Tripterygium wilfordii polyglycosides.

Discussion

Diabetes mellitus (DM) is a metabolic disease characterised by persistent hyperglycaemia accompanied by lipid and protein metabolic disorders[6]. Diabetic nephropathy (DN) is one of the most harmful complications of DM and is one of the main causes of chronic renal failure. It has been reported that tubulointerstitial fibrosis is mainly caused by the interaction of interstitial cells and inflammatory cell deposition, tubular cell reduction, and so on[7-8]. Moreover, DN is a natural immune and low-grade inflammatory disease. The inflammatory reaction caused by the expression of cytokines, especially TGF-beta 1 and NF-kappa B, is closely related to the occurrence and development of DN. Tripterygium wilfordii polyglycosides play a strong immunosuppressive role in the treatment of kidney diseases, leaving the normal immune system of the human body less damaged[9].

The TGF-β1 is a cytokine secreted by glomerular mesangial cells. It is the initiating hub of fibrosis formation and development. It can mediate the pathological process of glomerular fibrosis[10]. The TGF-β1 can inhibit the proliferation and differentiation of most kidney cells, changing the transition deposition of ECM in the glomerular mesangial region and accelerating the proliferative changes in renal fibrosis. Studies have shown that[11] the downstream signal molecules of the TGF-β1 and the classic Smad pathway include MAPK and NF-κB and other non-Smad signal pathways. The TGF-β1 has become the initiator of fibrosis. In addition, the NF-κB signalling pathway is a classical inflammatory response signalling pathway, which participates in innate immunity, inflammatory responses, fibrosis, and other biological effects[12]. It has been shown that the TGF-β1 may play a role in promoting liver fibrosis through the signalling pathway of NF-κB. Activating NF-κB may affect the downstream factors closely related to ECM synthesis and the NF-κBp65 formed by activation in the nucleus, thus promoting the occurrence and development of pulmonary fibres[13]. The results show that the expression of TGF-β1 and NF-κBp65 in the model group was significantly higher than that in the control group, and the levels of NF-κBp65 nRNA in the serum and kidney tissues were significantly higher than those in the control group. After TWP treatment, the expression of TGF-β1/NF-κBp65 decreased to different degrees compared with the model group, indicating that

the inflammatory response signalling pathways of TGF-β1/NF-κB were activated. Tripterygium wilfordii polyglycoside is an effective component of Tripterygium wilfordii, which contains a small amount of diterpenoids or alkaloids. It is commonly used in the treatment of glomerulonephritis, rheumatoid arthritis, and so on. It can significantly inhibit the proliferation of mesenteric cells, reduce the damage to the glomerular mechanical barrier, reduce glomerular fibrosis, alleviate glomerular microangiopathy, and delay the DN process[14-15]. In our previous experiments, we found that TWP could significantly reduce urinary protein levels in high- and medium-dose groups. After TWP treatment, the expression of TWP in high- and medium-dose groups was significantly lower than that in the model group and gradually decreased with the increase in the TWP dose. This suggests that TWP can inhibit renal fibrosis in DM patients, which may be related to its inhibition of inflammatory factors related to the TGF-β1/NF-κB signalling pathway.

In conclusion, the TGF-β1/NF-κB signalling pathway mediates the occurrence and development of renal fibrosis. Tripterygium wilfordii polyglycosides can improve diabetic renal fibrosis by inhibiting the TGF-β1/NF-κB signalling pathway in a dose-dependent manner.

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