Yi Qi Qing Re Gao-containing serum inhibits lipopolysaccharide-induced rat mesangial cell proliferation by suppressing the Wnt pathway and TGF-β1 expression

LIPING YANG[1*], XUEYAN SUN[2*], YONGLI ZHAN[1], HUIJIE LIU[2], YUMIN WEN[2], HUIMIN MAO[2], XI DONG[3] and PING LI[3]

[1]Department of Nephrology, Guang'Anmen Hospital of China Academy of Traditional Chinese Medicine Sciences, Beijing 100053; [2]Beijing University of Chinese Medicine, Chaoyang, Liaoning 100029; [3]Department of Pharmacology, Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, Beijing 100029, P.R. China

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Abstract. The aim of the present study was to investigate the effect of Yi Qi Qing Re Gao-containing serum (YQ-S) on rat mesangial cell (MC) proliferation and to investigate the underlying mechanism. MCs were divided into the control, lipopolysaccharide (LPS)-stimulated, YQ-S and fosinopril-containing serum (For-S) groups, and cultured for 48 h. An MTT assay was used to evaluate the proliferation of MCs. In addition, reverse transcription-quantitative polymerase chain reaction and western blot analysis were conducted to detect the expression levels of Wnt4, β-catenin and transforming growth factor (TGF)-β1 in MCs. The results indicated that YQ-S inhibited LPS-induced MC proliferation. The Wnt4 and TGF-β1 mRNA expression levels were reduced in the YQ-S group ($P<0.01$ or $P<0.05$). Furthermore, the Wnt4, β-catenin and TGF-β1 protein expression levels were suppressed in the YQ-S group ($P<0.01$ or $P<0.05$). Therefore, YQ-S appears to inhibit MC proliferation, and its mechanism may involve the inhibition of the Wnt signaling pathway and downregulation of TGF-β1 expression.

Introduction

Chronic kidney disease (CKD) is a major challenge for global public health care, due to its irreversible progression, low awareness and high cost (1). Due to the progressive aging of the general population, and the emerging epidemic of obesity and diabetes, CKD is now one of the three leading causes of mortality worldwide (1,2). Studies regarding the pathogenesis of CKD have predominantly focused on glomerular sclerosis and interstitial fibrosis, with disturbance of extracellular matrix (ECM) homeostasis (i.e. synthesis and degradation) a common factor (3). Mesangial lesions, caused by mesangial cell (MC) proliferation and accumulation of ECM, are universal morphological manifestations of renal diseases that involve the mesangium. There are numerous growth factors, cytokines and signaling pathways that are involved in the process of mesangial fibrosis, among which transforming growth factor-β1 (TGF-β1) is the most versatile, with functions in cell growth, apoptosis, proliferation, and ECM production (4). TGF-β1 is able to affect MC proliferation, and stimulate the synthesis and inhibit the degradation of ECM in an autocrine or paracrine manner, leading to relentless progression of glomerular sclerosis (5).

The Wnt signaling pathway is a key regulator of embryonic development, tissue homeostasis, cell apoptosis, proliferation and differentiation (6-8). There are 19 known Wnts, which mediate at least three divergent signaling pathways: The β-catenin dependent pathway (canonical Wnt pathway), the Ca^{2+}-pathway, and the planar cell polarity-pathway; the latter two pathways are also known as non-canonical Wnt pathways. In the canonical Wnt pathway, Wnt proteins bind to a receptor complex formed by Frizzled and low-density lipoprotein receptor-related protein 5 or 6, which leads to disassembly of the β-catenin destruction complex, which is composed of axin, adenomatous polyposis coli protein, and glycogen synthase kinase-3β (8). Disassembly of the β-catenin destruction complex hinders the normal phosphorylation and ubiquitination process of cytoplasmic β-catenin, allowing it to accumulate in the cytosol and translocate into the nucleus, where it stimulates the transcription of its target genes, including Twist, Snail, fibronectin, and matrix metalloproteinase-7 (9). The Wnt pathway has previously been demonstrated to be involved in renal tubular atrophy and interstitial fibrosis of an obstructed renal model (10,11), podocyte apoptosis and dysfunction, albuminuria and slit diaphragm

Correspondence to: Dr Ping Li, Department of Pharmacology, Institute of Clinical Medical Sciences, China-Japan Friendship Hospital, 2 Yinghua Dongjie, Beijing 100029, P.R. China
E-mail: pingli1234@yeah.net

*Contributed equally

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abnormalities (12-14), and high glucose-induced MC apoptosis (15,16). Interactions between Wnt/β-catenin and TGF-β signaling pathways have been shown to be important in the pathogenesis of fibrosis (17,18). In addition, manipulation of the Wnt pathway may rescue TGF-β1 mediated fibrosis.

Yi Qi Qing Re Gao (YQ) is a traditional Chinese medicine mixture developed by Zhan and Dai (19), which has been used for decades with satisfactory clinical effects. In a previous clinical study, YQ was demonstrated to reduce proteinuria, elevate serum albumin, decrease serum cholesterol, and ameliorate certain symptoms associated with chronic nephritis (19). Furthermore, a previous animal study in an adriamycin rat model suggested that YQ may be able to reduce glomerular mesangial collagen type IV, fibronectin and laminin content (20). Our recent study revealed that YQ was able to attenuate podocyte injury and inhibit vascular endothelial growth factor overexpression in a puromycin aminonucleoside rat model (21). However, the mechanisms underlying the effects of YQ on MC proliferation and glomerular sclerosis remain to be elucidated. The present study aimed to investigate the expression of Wnt pathway-associated molecules and TGF-β1 in LPS-stimulated MCs, and to determine the effects of YQ on this pathway, in order to provide evidence for the role of YQ in the prevention and treatment of glomerulosclerosis.

Materials and methods

Animals. A total of 40 healthy male Wistar rats (age, 7-8 weeks), weighing 250±20 g, were purchased from Beijing China Fukang Biological Technology Co., Ltd. (no. SCXK 2009-0007; Beijing, China). Rats were housed in the specific pathogen-free animal facility at the China-Japan Friendship Hospital (Beijing, China), with free access to water and standard rat chow. This study was approved by the Ethics Committee of the China-Japan Friendship Hospital and conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals (no. 2010-A10).

Cell line. A rat mesangial cell line was purchased from Peking Union Medical College (no. HBZY-1; Beijing, China).

Reagents and instruments. Minimum essential medium (MEM), streptomycin and ampicillin, phosphate-buffered saline (PBS) and trypsin were all purchased from GE Healthcare (Logan, UT, USA). In addition, fetal bovine serum (FBS) was obtained from Gibco Life Technologies (Carlsbad, CA, USA); lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (St. Louis, MO, USA); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was from Enzo Life Sciences, Inc. (Farmingdale, NY, USA); TRIzol reagent was from Invitrogen Life Technologies (Carlsbad, CA, USA); RevertAid™ First Strand cDNA Synthesis kit was from Fermentas, Thermo Fisher Scientific (Vilnius, Lithuania); Wnt4 goat polyclonal antibody (cat. no. sc-5214) was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA); mouse TGF-β1 monoclonal antibody (cat. no. ab64715) was from Abcam (Cambridge, UK); rabbit β-catenin monoclonal antibody (cat. no. 8480S) was from Cell Signaling Technology, Inc. (Danvers, MA, USA); goat anti-mouse IgG and goat anti-rabbit IgG antibodies were from Beijing Zhongshanjinqiao Biotechnology Co., Ltd (Beijing, China). The instruments used in the present study were as follows: ABI 2720 thermal cycler (Applied Biosystems Life Technologies, Foster City, CA, USA); AlphaImager TM2200 gel imaging system (Alpha Innotech Corporation, San Leandro, CA, USA); 1X51 inverted microscope (Olympus Corporation, Tokyo, Japan); and NAPCO CO_2 incubator (Thermo Fisher Scientific, Inc., San Jose, CA, USA).

Experimental drugs. YQ was obtained from Guang'Anmen Hospital (Beijing, China; certificate no. 98; Beijing Health & Drug no. 058). The mixture included: 72 g *Radix Astragali Mongolici* (Huang Qi), 54 g *Rhizoma Atractylodis Macrocephalae* (Bai Zhu), 36 g *Radix Ledebouriellae* (Fang Feng), 72 g *Flos Lonicerae* (Jin Yin Hua), 72 g *Fructus Forsythiae* Suspensae (Lian Qiao), 54 g *Herba Duchesneae Indicae* (She Mei), 180 g *Herba Hedyotis Diffusae* (Bai Hua She She Cao), 72 g *Poria Cocos* (Fu Ling), 125 g *Rhizoma Alismatis* (Ze Xie), 180 g *Herba Leonuri* (Yi Mu Cao), 180 g *Rhizoma Imperatae* (Bai Mao Gen) and 70 g *Rhizoma Dioscoreae Nippponicae* (Chuan Shan Long). Herbal medicines were prepared as follows: Water decoction for 30 min, followed by concentration of the supernatant under ordinary pressure, centrifugation at 2,000 x g for 15 min, and sterilization by filtration. The final standardized product consisted of 4.5 g crude medicine per milliliter. fosinopril tablets, containing fosinopril sodium, were provided by Sino-American Shanghai Squibb Pharmaceutical Ltd. (Shanghai, China).

Preparation of drug-containing sera. After three days of acclimation, 40 Wistar rats were divided at random into three groups, including the YQ (n=10), fosinopril (n=10) and normal (n=20) groups. Rats received an intragastric dose of YQ (5.7 g/kg, which corresponds to 20 times the adult clinical dose), fosinopril (1.67 mg/kg, which corresponds to 10 times the adult clinical dose), or an equal volume of distilled water, twice a day for seven days. Abdominal aortic blood (5-10 ml) was collected following the final drug administration. Blood samples were allowed to stand at 4˚C for 4 h, followed by centrifugation at 900 x g for 10 min at 4˚C. Sera were carefully extracted by suction and then incubated in a 56˚C water bath for 30 min to inactivate complements and antibodies present in the sera. Subsequent to 0.22 μm filter-sterilization, the sera were stored in sterile centrifuge tubes at -20˚C.

Recovery and passage of cells. Frozen rat MCs (HBZY-1) were removed from liquid nitrogen, and rapidly transferred to a 37˚C water bath for ~1 min until dissolved into a mixture of ice and water. MCs were subsequently transferred to a 25 cm^2 culture flask with 5 ml MEM containing 10% FBS, and 100 IU/ml streptomycin and ampicillin, and then placed in a 5% CO_2 incubator at 37˚C. Following overnight culture until the cells became adherent, the medium was changed. The medium was discarded when the MCs were in the logarithmic phase, and the cells were washed twice with PBS, followed by the addition of 1 ml trypsin (0.25%). When the cells exhibited a round shape, culture medium was immediately added to terminate the digestion. Subsequently, the cell suspension was transferred to a new culture flask containing 10% FBS-containing MEM, and placed in an incubator with 5% CO_2 at 37˚C for 3-5 days

Table I. Polymerase chain reaction primers and reaction parameters.

Primer	Primer sequence	Fragment length (bp)	Annealing temperature (˚C)	Cycles
Wnt4	F 5'-CGGGAAGGTGGTGACACAAG-3' R 5'-GCTCGCCAGCATGTCTTTAC-3'	375	58	34
β-catenin	F 5'-AATGGCTTGGAATGAGACTG-3' R 5'-AGCCCATCAACTGGATAGTC-3'	198	56	30
TGF-β1	F 5'-TACCATGCCAACTTCTGTCTG-3' R 5'-CACGATCATGTTGGACAACTG-3'	204	58	29
Cyclophilin B	F 5'-GTGGTTTTCGGCAAAGTTCTG-3' R 5'-GGCAAAGGGTTTCTCCACTTC-3'	147	56	29

TGF-β1, transforming growth factor-β1; F, forward; R, reverse.

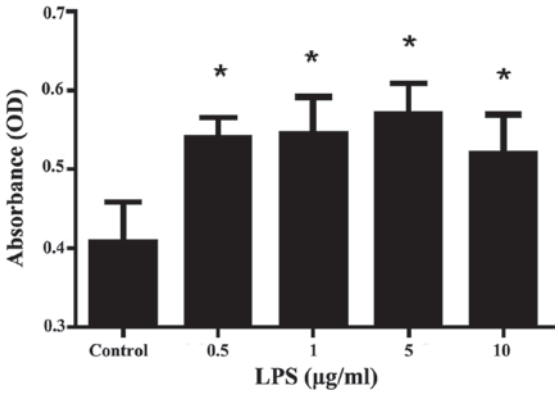

Figure 1. Different concentrations of LPS on mesangial cell proliferation (n=3). *P<0.01 vs. control group. OD, optical density; LPS, lipopolysaccharide.

until they reached 80-100% confluence. The 5th generation of MCs was used for subsequent experimentation.

Experimental design. Cells of the 5th generation were transferred into 96-well plates ($3x10^3$/well), and incubated with MEM supplemented with 5% FBS and 20% normal rat serum for 24 h. Subsequently, the cells were incubated with MEM containing 2% FBS for 24 h, in order to be synchronized at G_0 phase. The cells were divided into: Control group (incubated with MEM + 5% FBS + 20% normal rat serum), the LPS-stimulated (LPS) group (incubated with MEM + 5% FBS + 20% normal rat serum + 5 μg/ml LPS), the YQ containing serum (YQ-S) group (incubated with MEM + 5% FBS + 20% YQ-S + 5 μg/ml LPS), the fosinopril containing serum (For-S) group (incubated with MEM + 5% FBS + 20% For-S + 5 μg/ml LPS). There were 6 wells for each group.

Effect of LPS and drug-containing serum on rat MC proliferation. Once the cells were synchronized at G_0 phase after 24 h, the supernatant was removed and 200 μl medium supplemented with 5% FBS and various concentrations of LPS (0.0, 0.5, 1.0, 5.0 and 10.0 μg/ml), and YQ-S or For-S (5, 10 and 20%) were added to each well (n=3, triplicate wells). The cells were further incubated for 48 h, and subsequently 150 μl MTT (5 mg/ml) was added to each well. Following incubation at 37˚C for 4 h, the supernatant was discarded carefully, and 150 μl DMSO was added to each well. Following shaking at room temperature for 10 min, the optical density values were measured at 492 nm using a microplate absorbance reader (Model 680; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each experiment was repeated three times.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for the detection of Wnt4, β-catenin and TGF-β1 mRNA expression levels. MCs in the logarithmic growth phase were digested and suspended, and $1x10^5$ cells were seeded in a 100 mm culture dish and incubated for 24 h in order to reach adherence. After 48 h of stimulation according to the various groups, the cells were lysed with TRIzol to extract the total RNA, which was then reverse transcribed into cDNA. PCR amplification primers were synthesized by Beijing Qing Ke New Industrial Biotechnology Co., Ltd. (Beijing, China). Primer sequences and reaction conditions are presented in Table I. PCR was performed in triplicate in a final volume of 12.5 μl: 6.25 μl PCR mix (Beijing ComWin Biotech Co., Ltd., Beijing, China), 2 μl diluted cDNA products, 1 μl of each paired primer, and 3.25 μl deionized water. PCR was carried out using an ABI 2720 thermal cycler (Applied Biosystems Life Technologies). Cycling conditions were as follows: Initial denaturation for 2 min at 94˚C, followed by 29 cycles (for cyclophilin B and TGF-β1), 30 cycles (for β-catenin) or 34 cycles (for Wnt4) of denaturation for 30 sec at 94˚C, extension for 1 min at 72˚C, and a final elongation for 10 min at 72˚C. PCR products were loaded onto a 1.2% agarose gel and electrophoresis was performed at 100 V constant voltage for 30 min. Gel images were analyzed using ImageJ 1.40g software (National Institutes of Health, Bethesda, MD, USA), which was used to determine each gray value of the target band. Relative quantities of each target gene were represented as the ratio of the gray values of target to that of cyclophilin B.

Western blot analysis of Wnt4, β-catenin and TGF-β1 protein expression levels. Following 48 h of stimulation according to the grouping aforementioned, the MCs were collected and lysed with cell lysis buffer (150 mM NaCl, 1 mM EDTA, 20 mM Tris HCl, pH 7.4) and 1X protein

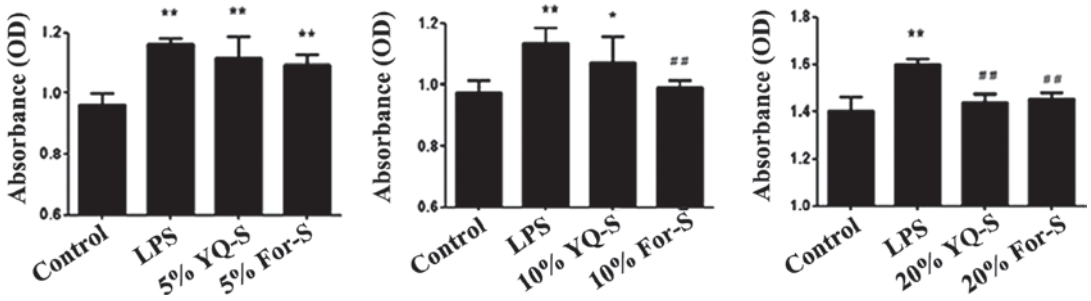

Figure 2. Different concentrations containing serum on proliferation of mesangial cells. *P<0.05 and **P<0.01 vs. control group; ##P<0.01 vs. LPS stimulation group. OD, optical density; LPS, lipopolysaccharide; YQ-S, Yi Qi Qing Re Gao-containing serum; For-S, fosinopril-containing serum.

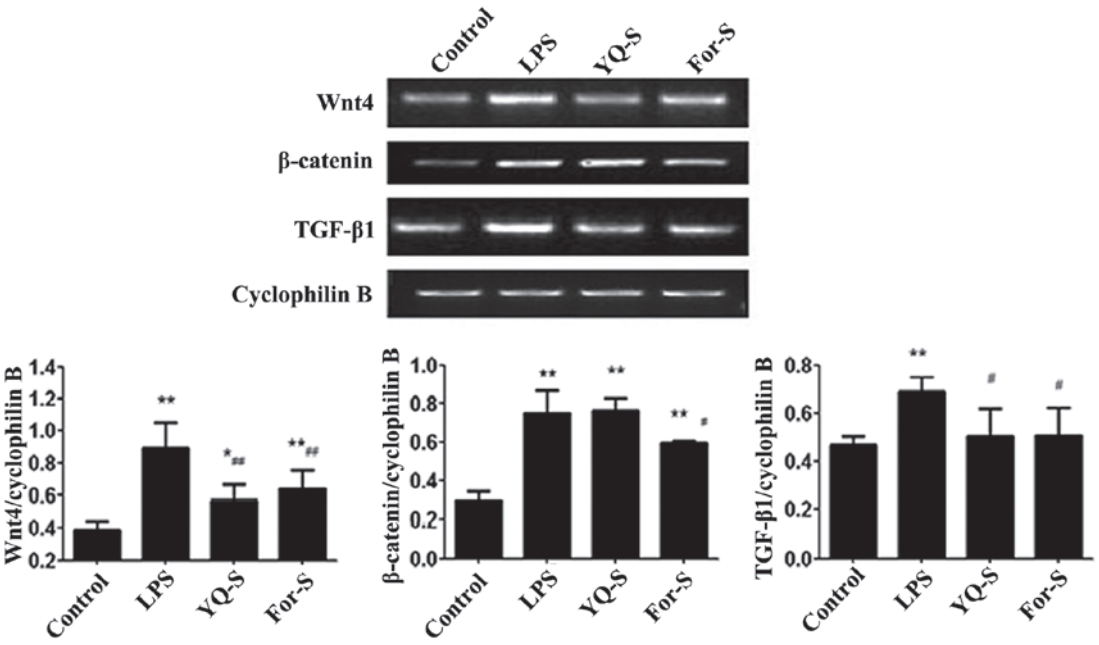

Figure 3. Wnt4, β-catenin and TGF-β1 mRNA expression in rat mesangial cells. *P<0.05 and **P<0.01 vs. control group; #P<0.05 and ##P<0.01 vs. LPS stimulation group. LPS, lipopolysaccharide; YQ-S, Yi Qi Qing Re Gao-containing serum; For-S, fosinopril-containing serum; TGF-β1, transforming growth factor-β1.

inhibitor cocktail for extraction of total proteins. The protein concentrations of the samples were determined by Bradford assay (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Samples containing 60 μg proteins were loaded onto 10% SDS-PAGE gel and separated by electrophoresis. The initial electrophoresis with condensed gel was conducted at 80 V for 25 min, followed by electrophoresis with separation gel at 150 V for 50 min. The proteins were then electrotransferrd to a polyvinylidene fluoride membrane (EMD Millipore, Billerica, MA, USA), and the membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 2 h. The membrane was then incubated at 4˚C overnight with the primary antibodies against Wnt4 (1:1,000), β-catenin (1:1,000) and TGF-β1 (1:2,000), and was subsequently washed three times with TBST for 10 min. Horseradish peroxidase-conjugated second antibodies (1:3,000) were then added. Following 1 h incubation with agitation, the membranes were washed three times with TBST for 10 min. Following visualization in the dark for 5 min in a Tanon 4500 chemical imaging system, the images were analyzed using ImageJ 1.40g software. Relative expression levels of the target proteins were quantified as the ratio of the target bands to that of β-actin.

Statistical analysis. Experimental data were analyzed using SPSS statistical software, version 17.0 (SPSS, Inc., Chicago, IL, USA). Measurement data are expressed as the mean ± standard deviation. Measurement data among different groups were compared using single-factor analysis of variance. Groups were compared using the least significant difference test and P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of LPS on rat MC proliferation. Compared with the control group, different concentrations of LPS (0.5, 1.0, 5.0 and 10 μg/ml) were able to stimulate rat MC proliferation to various degrees (P<0.01), among which the effect of 5.0 μg/ml LPS was the most evident. Combined with the results of our previous study (22), LPS 5.0 μg/ml was selected as the stimulation dose for the subsequent experiments (Fig. 1).

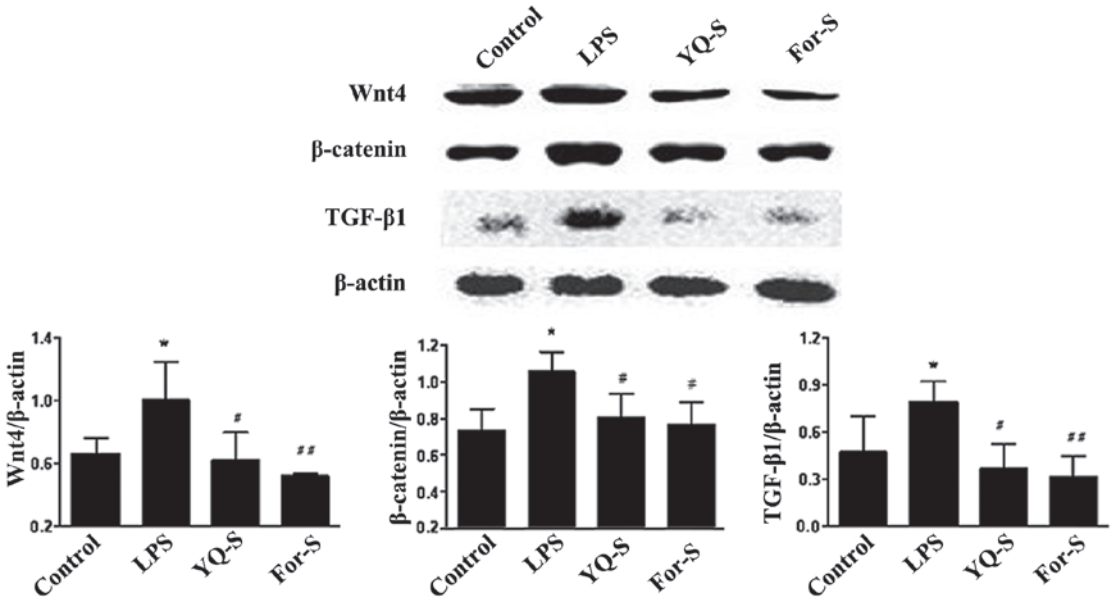

Figure 4. Wnt4, β-catenin and TGF-β1 protein expression levels in rat mesangial cells. *P<0.05 vs. control group; #P<0.05 and ##P<0.01 vs. LPS stimulation group. LPS, lipopolysaccharide; YQ-S, Yi Qi Qing Re Gao-containing serum; For-S, fosinopril-containing serum; TGF-β1, transforming growth factor-β1.

Effect of YQ-S on rat MC proliferation. The MTT assay results indicated that, compared with the LPS group, 5% YQ-S and 5% For-S exerted no significant inhibition on MC proliferation. Following treatment of the MCs with 10% For-S or 10% YQ-S, only For-S exerted a significant inhibitory effect on LPS-induced MC proliferation (P<0.01). In the MCs that received a 20% serum containing intervention, both YQ-S and For-S significantly inhibited MC proliferation (P<0.01). Therefore, 20% serum was selected as the appropriate dose for the subsequent intervention experiments (Fig. 2).

Effect of YQ-S on LPS-stimulated Wnt4, β-catenin and TGF-β1 expression. After LPS stimulation for 48 h, the MCs were collected for RT-qPCR and western blot analysis. The results indicated that, compared with control group, the expression levels of Wnt4, β-catenin and TGF-β1 were significantly increased (P<0.01 or P<0.05). Following stimulation of the MCs with the drug-containing serum for 48 h, RT-qPCR results indicated that, compared with LPS group, the Wnt4 and TGF-β1 mRNA expression levels were significantly reduced in the YQ-S and For-S groups (P<0.01 or P<0.05), while β-catenin mRNA expression was significantly reduced in the For-S group (P<0.05). Western blot analysis revealed that, compared with the LPS stimulation group, Wnt4, β-catenin and TGF-β1 protein expression levels were significantly reduced in the YQ-S and For-S groups (P<0.01 or P<0.05; Figs. 3 and 4).

Discussion

MCs are located in the central stalk of glomeruli and have various fundamental roles, including maintaining the structural backbone of the glomerular tuft, regulating capillary blood flow and thus glomerular filtration rate, generation of the mesangial matrix, and manipulation of immune complexes (5). Injuries to MCs, predominantly cell proliferation, hypertrophy, apoptosis, and ECM deposition, are pathological hallmarks of a wide spectrum of glomerular diseases, which lead directly to progressive renal failure (23). The mechanisms associated with MC injury have recently garnered extensive attention. The Wnt signaling pathway is an evolutionarily conserved pathway across species, which is closely associated with numerous kidney diseases, including ischemic renal injury, acute kidney injury (AKI), diabetic nephropathy, renal interstitial fibrosis and renal tumors. In ischemia-reperfusion injury of AKI, β-catenin ameliorates tubular epithelial cell apoptosis via activation of the Akt pathway, induction of survival and suppression of p53 (24). Activation of the canonical Wnt pathway contributes to obstructive renal injury, and the Wnt antagonist Dickkopf-1 (DKK-1) protects against renal fibrosis by inhibiting Wnts (10). In podocyte injury and proteinuric kidney diseases, the Wnt pathways are essential regulators and potential therapeutic targets (13,14). Studies regarding the role of the Wnt signaling pathway in MCs have mainly focused on hyperglycemic conditions (15,16,25,26). Lin *et al* (15) observed that in diabetic nephropathy, the Wnt/β-catenin signaling pathway serves a crucial role in the regulation of MC proliferation. *In vitro*, high glucose-induced apoptosis of MCs is accompanied by reduced expression of Wnt4 and Wnt5a. In addition, transfection of MCs with Wnt4, Wnt5a or persistent activation of β-catenin, was able to inhibit high glucose-induced mesangial apoptosis. Furthermore, treatment with simvastatin restored Wnt4, Wnt5a and β-catenin expression in MCs and attenuated diabetic kidney injuries both *in vivo* and *in vitro* (16). In addition, it has been demonstrated that abrogation of oxidative stress may restore Wnt5a/β-catenin signaling and attenuate high glucose-induced MC apoptosis (25). DKK-1 expression was also increased in these same conditions, and mediated c-Jun, TGF-β1, and fibronectin expression, whereas stable β-catenin expression alleviated DKK-1-induced profibrotic factors, indicating that β-catenin

may be a potential therapeutic target of MC dysfunction of diabetic nephropathy (26). In immune-mediated MC injury, the Wnt pathway has been demonstrated to be involved in the anti-Thy-1.1 rat model of glomerulonephritis (27), and development of lupus nephritis (28). However, there is currently scarce *in vitro* evidence regarding Wnts and β-catenin expression in LPS-stimulated MCs. As demonstrated in the present study, following stimulation of MCs with LPS for 48 h, the expression levels of Wnt4 and β-catenin were significantly elevated, indicating that both the canonical and non-canonical Wnt pathways were activated, and treatment with YQ-S was able to attenuate MC proliferation by inhibiting the Wnt signaling pathways. Further studies are required to explore the mechanisms underlying this phenomenon. Previous studies have indicated that TGF-β1 is a key mediator of glomerular sclerosis and MC injury (4,29,30). Crosstalk between TGF-β1 and the canonical Wnt signaling pathway has recently garnered much attention. In cultured MCs, high glucose levels increased TGF-β1 and fibronectin expression, and reduced Wnt4, Wnt5a and β-catenin expression. Restoring Wnt4, Wnt5a and cytosolic β-catenin significantly abrogated TGF-β1 expression, and exogenous inhibitors of the Wnt pathway also significantly alleviated TGF-β1-induced renal fibrosis, thus indicating that the Wnt pathway, particularly the β-catenin-associated pathway, is an important regulator of TGF-β1 expression in MCs (18). Furthermore, it has been demonstrated that, in experimental fibrosis, TGF-β1 may decrease DKK-1 levels in a p38-dependent manner, thus stimulating the canonical Wnt pathway (17). Wnt11 was identified by a gene screen study as the direct target of the stimulated TGF-β1/Smad3 pathway in renal epithelial cells, and further transferred its signals by the c-Jun N-terminal kinase (JNK) pathway (31). Zu *et al* previously demonstrated that LPS induced MC proliferation and upregulated TGF-β1, c-jun, and c-fos (22). The present study demonstrated that TGF-β1 expression was elevated after 48 h incubation with LPS, as was Wnt4 and β-catenin, thus indicating that the Wnt/β-catenin/TGF-β1 pathways are fundamental for LPS-induced MC proliferation; however, whether Wnt/β-catenin or TGF-β1 is upstream of this process requires further study.

The effectiveness of YQ for treating chronic nephritis has been indicated by previous studies in animal models and patients (19-21). The pharmacological mechanisms underlying the effects of this mixture have yet to be elucidated. There are 12 components of YQ, and many of these have previously been shown to exert regulatory effects on the Wnt pathway. *Radix Astragali Mongolici* (Huang Qi) is the main component of YQ, and Astragaloside IV (AS-IV) is the major active ingredient. In the unilateral ureteral occlusion (UUO) model of renal interstitial fibrosis, AS-IV was able to attenuate renal fibrosis and restore renal function by inhibiting TGF-β1, connective tissue growth factor and α-smooth muscle actin (SMA) expression, Smad2/3 phosphorylation, and collagen matrix expression, and upregulating Smad7. Similar results have been detected in TGF-β1-stimulated rat renal NRK-49F fibroblasts. Knockdown of Smad7 significantly abrogated these effects, indicating that the renal protective effects of AS-IV occur predominantly via Smad7 and therefore the TGF-β1/Smads pathway (32). Previous studies have demonstrated that there are synergistic effects of AS-IV and ferulic acid on TGF-β1, α-SMA, and p-JNK expression in the same rat and cell models of renal fibrosis (33). Astragaloside has also been shown to exert inhibitory effects on TGF-β1, Wnt4, Wnt5a, Frizzled-2, -3, -6 and β-catenin expression in a cholestatic liver fibrosis rat model (34). *Flos Lonicerae* (Jin Yin Hua) together with *Fructus Forsythiae* Suspensae (Lian Qiao) have anti-inflammatory effects in rat models of chronic obstructive pulmonary disease, via inhibition of tumor necrosis factor-α, TGF-β1 and interleukin-1β expression, and there were synergistic effects of those two compounds with *Radix Platycodon* (35). In the UUO rat model, leonurine, the active component of *Herba Leonuri* (Yi Mu Cao) ameliorated renal tubulointerstitial fibrosis and inflammation via the TGF-β/Smad3 and nuclear factor-κB pathway, indicating the potential renoprotective effect of leonurine (36). The regulatory effects of YQ in LPS-stimulated MCs via inhibition of the Wnt signaling pathway and TGF-β1 expression may be due to the aforementioned mechanisms of the active components in YQ.

In conclusion, the results of the present study demonstrated that LPS is able to promote the proliferation of MCs and induce the increased expression of Wnt4, β-catenin and TGF-β1. In addition, a positive correlation was detected between Wnt and TGF-β1. Furthermore, YQ-S and For-S treatments were shown to reduce the mRNA and protein expression levels of Wnt4, β-catenin and TGF-β1. Future studies are required in order to investigate the mechanisms underlying the effects of YQ-S and its component herbs on the Wnt and TGF-β1 signaling pathways.

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