Wenyang Huazhuo Fang exerts transient receptor potential cation channel subfamily C member-dependent nephroprotection in a rat model of doxorubicin-induced nephropathy

An Peng, Dong Sheng, Li Xingyao, Cai Zimo, Ye Bingyu, Zhang Aijun, Shi Xingmin, Wu Xili

An Peng, Li Xingyao, Cai Zimo, Ye Bingyu, Wu Xili, Department of Integrated Chinese Traditional and Western Medicine, the Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an 710004, China

Dong Sheng, Department of Nephropathy, the Affiliated Hospital of Shaan Xi University of Chinese Medicine, Xianyang 712023, China

Zhang Aijun, Department of Pharmacy, the Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an 710082, China

Shi Xingmin, Department of Immunology, Xi’an Jiaotong University Health Science Center, Xi’an 710061, China

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Correspondence to: Prof. Wu Xili, Department of Integrated Chinese Traditional and Western Medicine, the Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an 710004, China. wuxili1984@163.com

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Abstract

OBJECTIVE: To determine the effect of Wenyang Huazhuo Fang (WHF), a Traditional Chinese Medicine decoction, on renal function in a rat model of doxorubicin-induced nephropathy, and to elucidate the underlying mechanism.

METHODS: Sprague-Dawley rats were randomly divided into six groups: control, doxorubicin-nephropathy, and prednisone-treated (6.45 mg·kg⁻¹·d⁻¹) doxorubicin nephropathy groups, as well as high- (7.26 g·kg⁻¹·d⁻¹), medium- (2.42 g·kg⁻¹·d⁻¹), and low-dose (0.81 g·kg⁻¹·d⁻¹) WHF-treated doxorubicin-nephropathy groups. The nephropathy rat model was established by two tail vein injections of doxorubicin, followed by prednisone or WHF treatment for 8 weeks. Body weights were monitored and urinary protein was measured every 2 weeks. After the end of the treatment period, the rats were euthanized. Serum biochemical indicators were determined and renal morphological alterations were assessed using histological staining. The expression of transient receptor potential cation channel subfamily C member 6 (TRPC6), stromal interaction molecule 1 (STIM1), and calcium release-activated calcium channel protein 1 (Orai1) was detected using western blotting, and their mRNA levels were examined using quantitative real-time reverse transcription-polymerase chain reaction.

RESULTS: WHF treatment was found to significantly ameliorate weight loss, proteinuria, hypalbuminemia, and dyslipidemia in doxorubicin-nephropathy rats. The protein and mRNA levels of TRPC6, STIM1, and Orai1 were partially, but significantly suppressed by prednisone or WHF treatment.

CONCLUSION: Treatment with WHF significantly ameliorates renal injury in a rat model of doxorubicin-induced nephropathy, which could be at least partially related to repression of the TRPC6 pathway.
Keywords: Nephropathy; Doxorubicin; TRPC6 cation channel; Stim1 protein, rat; Orai1 protein, rat; Wenyang Huazhuo Fang

INTRODUCTION

Nephrotic syndrome is caused by increased permeability of the glomerular capillary wall for proteins, and is characterized by heavy proteinuria, possibly accompanied by hypoproteinemia, hypercholesterolemia, lipiduria, and edema. Adult nephrotic patients might suffer from life-threatening thromboembolic complications, while children with nephrotic syndrome are at risk mainly from infectious complications. Persistent nephrotic syndrome can exacerbate negative nitrogen balance and malnutrition, accelerating atherosclerosis and progression to end-stage renal failure. Remission of nephrotic syndrome can be commonly achieved by either causative agent elimination or by immunosuppressive treatment. Extensive data strongly implicate podocytes as promising targets for the treatment of nephrotic syndrome. Podocytes, which are terminally differentiated cells in the renal glomerulus, are essential for the integrity of the kidney filter. Based on their intricate structure including foot processes, podocytes form actin-driven membrane extensions, the loss of which can lead to chronic kidney disease development, and ultimately renal failure. Podocytes can respond to glomerular hypertension and other mechanical stretches. However, the exact mechanisms of mechanoreception and mechanotransduction are yet to be elucidated. Evidence has shown that transient receptor potential cation channel subfamily C member 6 (TRPC6) plays an important role in the reception and transduction of mechanical stress. Winn et al.1 reported that a mutation in TRPC6 causes familial focal segmental glomerulosclerosis. The dynamic interaction of endogenous TRPC6 with either calcium release-activated calcium channel protein 1 (Orai1) or stromal interaction molecule 1 (STIM1) is involved in store-operated calcium entry.2 Orai channels are calcium-selective ion channels that are activated upon depletion of internal calcium stores. Meanwhile, STIM1 proteins sense the decreased calcium concentration in the endoplasmic reticulum.3 Upon depletion of the calcium stores, STIM1 proteins cluster and relocate near the plasma membrane, where they activate Orai1 via protein-protein interactions.4,5 Wenyang Huazhuo Fang (WHF) is a Traditional Chinese Medicine (TCM) formula comprising eleven herbs, namely Fuzi (Radix Aconiti Lateralis Preparata), Huangqi (Radix Astragali Mongolici), Shanzhuyu (Fructus Macrocarpi), Jingyingzi (Fructus Rosae Laevigatae), Qianshi (Semen Euryales), Dahuang (Semen Euryales), Guizhi (Ramus Cinnamomi), Fuling (Poria), and Cheqianzi (Semen Plantaginis).

Based on TCM principles that have evolved over the years of practice, this decoction has been applied to treat nephrotic syndrome and proteinuria. The long-term clinical practice in the Department of TCM, the Second Affiliated Hospital (Xibei Hospital) of Xi’an Jiaotong University, has supported the protective effect of WHF against nephrotic syndrome. However, preclinical research in this field is still sparse, and the underlying mechanism remains unclear. The pharmacological effect of individual herbs, rather than the whole formula, has been previously examined. Ryu et al.7 demonstrated that Dahuang extract has a strong inhibitory activity on oxidative stress, which plays a key role in the pathogenesis of renal diseases. Gao et al.8 reported antitumor and immunostimulating activities of water-soluble polysaccharides from Fuzi (Radix Aconitii Lateralis Preparata). Lee et al.9 showed immunomodulatory effects of aqueous-extracted Huangqi (Radix Astragali Mongolici) in methotrexate-treated spleen cells. In addition, Ryu et al.10 found that Huangqi exerts an anti-inflammatory effect by decreasing nitric oxide production in zymosan air-pouch mice. You et al.11 showed that the aqueous extract of Huangqi ameliorates proteinuria in rats with doxorubicin (Adriamycin)-induced nephropathy via inhibition of oxidative stress and endothelial nitric oxide synthase. The current study demonstrated that WHF treatment for 8 weeks ameliorated proteinuria, hypoalbuminemia, and lipid metabolism disorder in a rat model of doxorubicin nephropathy, and implicated TRPC6 pathway as the underlying mechanism. This study could provide useful information for the treatment of nephrotic syndrome using TCM formulas.

MATERIALS AND METHODS

Animals and experimental design

This study was approved by the Animal Ethics Committee of Xi’an Jiaotong University (Approval No. SCXK2012-003). Sixty male, specific-pathogen-free-grade Sprague-Dawley (SD) rats [(200±20) g] were purchased from the Experimental Animal Center in Xi’an Jiaotong University, Shaanxi Province, China. All rats were housed in a temperature-controlled room (25±3 °C) with a relative humidity of 40% to 60% and 12 h/12 h light-dark cycles, with free access to food and water. The rats were randomly divided into six groups (n=10 per group): control (Con); doxorubicin-nephropathy (Adr); prednisone-treated (6.45 mg·kg⁻¹·d⁻¹) doxorubicin-nephropathy (Pre) groups; as well as high-(7.26 g·kg⁻¹·d⁻¹, WH), medium-(2.42 g·kg⁻¹·d⁻¹, WM), and low-dose (0.81 g·kg⁻¹·d⁻¹, WL) WHF-treated doxorubicin-nephropathy groups. To establish the doxorubicin-nephropathy model, the rats were injected with doxorubicin (Zhejiang Hisun Pharmaceutical Co., Ltd., Taizhou, China) twice through the tail vein.
once at a dose of 4 mg/kg followed by 3.5 mg/kg a week later, as previously described.19 The rats in the Con group were injected with vehicle (0.9% saline). After model establishment, the rats in the Wh, Wm, and WI groups were administered WHF decoction at the indicated dosages by gavage (2 mL per day) for 8 weeks. All experiments were performed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals by NIH.

Preparation of WHF
WHF used in the current study comprised 11 herbs, namely 15 g of Fuzi (Radix Aconiti Lateralis Preparata), 15 g of Huangqi (Radix Astragali Mongolici), 15 g of Shanzhuuyu (Fructus Macrocarpini), 45 g of Jingyinzi (Fructus Rosae Laevigatae), 30 g of Qianshi (Semen Eu- ryales), 10 g of Dahuang (Radix Et Rhizoma Rhei Pal- mati), 15 g of Zelan (Herba Lycii Hirti), 15 g of Chuanxiong (Rhizoma Chuanxiong), 25 g of Guizhi (Ram- ulus Cinnamomi), 15 g of Fuling (Poria), and 20 g of Cheqianzhi (Semen Plantaginis) (Beijing Tong Ren Tang Group Co., Ltd., Beijing, China). The decoction was made according to TCM principles. In brief, all the herbs were mixed and then extracted with 500 mL of water at 100 °C for 1 h twice. The supernatants from each extraction were then combined. The final dose was determined by the body surface area (A = K*W2/3, K = 0.0886, W = (220 ± 20) g). WHF was developed by a TCM physician, Qiao Chenling, based on her clinical experience in treating nephrotic syndrome. Prednisone, an immunosuppressive agent, was used as a positive control throughout the experiment.

Body weight monitoring and biochemical parameter measurement
The body weight was measured every 2 weeks. The rats were housed individually in metabolic cages (Fengshi, China) for 24-h urine collection on weeks 0, 2, 3, 5, and 9. Urinary protein was detected by the pyrogallol red method.

After the end of the treatments at week 8, the rats were euthanized, and blood samples were collected from the abdominal aortas, followed by centrifugation at 3000 ×g for 15 min. Serum was obtained for the detection of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), blood urea nitrogen (BUN), total protein, and albumin. Examination of the above biochemical indicators was performed using an automatic biochemistry analyzer (AU2700, Olympus, Japan).

Histological analysis
The kidneys were excised for immunohistochemical ex- amination. The remaining kidney tissue was fixed in 4% paraformaldehyde solution and embedded in paraf fin. Sections of 3-μm thickness were cut, stained with hematoxylin and eosin (HE) and Masson trichrome, and observed under a light microscope (TS100, Nikon Instruments Inc., Japan). Samples of renal cortex were fixed in 2.5% glutaraldehyde and embedded in epoxy resin for electron microscopy examination (H-600, Hit- achi Ltd., Japan). The remaining renal cortex tissues were frozen in liquid nitrogen and stored at −80 °C for subsequent molecular studies.

Western blotting detection of TRPC6, STIM1, and ORAI1 in the rat renal cortex
The renal cortex of rats was frozen in liquid nitrogen, ground into powder, and protein lysates were generated (Sanbio, Beijing, China). After centrifugation at 16009.2 ×g for 15 min at 4 °C, the supernatants were transferred into new tubes and the protein content was determined by the Bradford assay. Samples containing 30 μg of protein were resolved on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and electrotransferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% nonfat skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBST) for 2 h, followed by overnight incubation at 4°C with the following primary antibodies: TRPC6 1: 500, STIM1 1: 1000, ORAI1 1: 300 (Abcam, USA); β-actin 1: 1000 (CMCTAG, USA). After rinsing with TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (1: 5000) (Zhuangzhi, Xi’an, China). After rinsing with TBST, each membrane was imaged with a G: BOX gel imaging and analysis system (Syngene, UK) using ECL Western Blotting Detection Reagent (Millipore, billerica, MA, USA). Band densities were analyzed using Quantity One Image Software (Bio-Rad, Hercules, CA, USA).

Quantitative reverse transcription-polymerase chain reaction
Total RNA samples were extracted from the kidney by using the RNAfast1000 Total RNA extraction kit (Pioneer Biotechnology, Xi’an, China), according to the manufacturer’s protocol. RNA purity was evaluated by measuring the ratio of A260/A280. Real-time reverse transcription-polymerase chain reaction (RT-qPCR) was performed using the PrimeScript RT Master Mix (Takara, Japan). The total reaction volume was 10 μL, and the reaction consisted of an initial denaturation step (10 min at 95 °C), followed by 40 cycles of denaturation (15 s at 95 °C), annealing (30 s at 60 °C), and extension (30 s at 72 °C). β-actin was used as an internal control, and the data were analyzed by StepOne™ software V2.1. The primers used in RT-qPCR are listed in Table 1.

Statistical analysis
Data are expressed as the mean ± standard error of mean (SEM). Comparisons between groups were evaluated using Student’s t-test by GraphPad Prism 7.01 (GraphPad Software Inc., La Jolla, CA, USA), and results were considered statistically significant at P values < 0.05.
RESULTS

**WHF treatment significantly inhibits the weight loss induced by doxorubicin injection in rats**

All the animals in the Con group exhibited normal physical appearance, with smooth fur and gradual weight gain. In contrast, the rats in the doxorubicin-nephropathy groups exhibited reduced activity, slower reactions, chills, and a tendency to huddle together, as well as different degrees of diarrhea and weight loss. Body weights were monitored every 2 weeks until the animals were euthanized. Compared to the Con group, the Adr group showed significantly lower average body weight at 8 weeks after doxorubicin injections (Figure 1A). The nephropathy rats treated with prednisone or WHF exhibited significantly higher average body weights than those without treatment (Figure 1A). There was no significant difference in average body weight between prednisons-treated group and medium- or low-dose WHF-treated group; however, the rats in the Wh groups had significantly higher average body weights than that in the Pre group (Figure 1A). These results suggest that WHF treatment for 8 weeks can significantly ameliorate the weight loss induced by doxorubicin nephropathy rats.

**WHF treatment significantly ameliorates proteinuria induced in doxorubicin-nephropathy rats**

Proteinuria is an important clinical manifestation in many renal diseases. The results show that the rats acquired severe proteinuria after two doxorubicin injections, with 24-h urinary protein of (53.8 ± 2.0) mg at week 2 and (104.4 ± 3.2) mg at week 3. Compared with the Adr group, 8-week treatment with prednisone or 3 doses of WHF significantly ameliorated the 24-h urinary protein levels (Figure 1B). Moreover, doxorubicin-nephropathy rats exhibited significantly higher levels of BUN than the Con group did (Figure 2C). It was found that treatment with prednisone or medium/ high doses of WHF significantly reduced the BUN level compared with the Adr group (Figure 2C). These results suggest that WHF treatment significantly ameliorates proteinuria induced in doxorubicin-nephropathy rats.

**WHF treatment significantly attenuates hypoalbuminemia induced by doxorubicin injection in rats**

As previously described,23 doxorubicin-induced nephrotic syndrome is accompanied by dyslipidemia, reduced serum albumin, and increased urinary protein. Eight weeks after doxorubicin injection, the rats in the Adr group showed significantly lower levels of total protein and serum albumin than those in the Con group (Figure 2A, 2B). Treatment with prednisone or any WHF dose significantly attenuated hypoalbuminemia and low levels of total protein compared with the Adr group (Figure 2A, 2B).

**WHF treatment significantly attenuates dyslipidemia induced by doxorubicin injection in rats**

As shown in Figure 2, the rats with untreated doxorubicin nephropathy showed severe dyslipidemia. The serum TC, TG, and LDL levels significantly increased compared with those in the Con group (Figure 2E, 2F). Treatment with prednisone or any dose of WHF significantly attenuated dyslipidemia in nephrotic rats (Figure 2D-2F). Notably, WHF exhibited a dose-dependent pattern in the normalization of TG levels. These results suggest that WHF treatment can significantly mitigate proteinuria and other complications in doxorubicin-nephropathy rats.

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**Table 1 Primers used in this study**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Forward (5’ to 3’)</th>
<th>Reverse (5’ to 3’)</th>
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<tr>
<td>TRPC6</td>
<td>TGGGATGTGAGGAGGA</td>
<td>AAGCAGGGACTTTGGGAC</td>
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<tr>
<td>STIM1</td>
<td>GGAAGGGTGAGAAGGAA</td>
<td>GAAAGGCGAGAGGGAAA</td>
</tr>
<tr>
<td>ORAI1</td>
<td>CCGCAACAGCAGCAACA</td>
<td>CCGTAAAGTGAGCAGCAG</td>
</tr>
<tr>
<td>β-Actin</td>
<td>GGAGATTACTGGCCCTGCTCTA</td>
<td>GACTCACTGGTACTCCTGCTG</td>
</tr>
</tbody>
</table>

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**Figure 1 Effect of Wenyang Huazhuo Fang on body weight and 24-h urine protein in rats induced by doxorubicin injection**

A: body weight; B: 24-h urine protein. Con: control group; Adr: doxorubicin-nephropathy group; Pre: prednison-treated (6.45 mg · kg⁻¹ · d⁻¹, 8 weeks in total); Wi: low-dose (0.81 g · kg⁻¹ · d⁻¹, 8 weeks in total); Wm: medium-dose (2.42 g · kg⁻¹ · d⁻¹, 8 weeks in total); Wh: high-dose (7.26 g · kg⁻¹ · d⁻¹, 8 weeks in total). Values are mean ± standard error of mean. **P** < 0.01 vs Adr; **P** < 0.01 vs Con.
WHF treatment significantly mitigates morphological damage induced by doxorubicin injection in rats

As shown by HE staining (Figure 3), rats with doxorubicin-induced nephropathy developed a moderate expansion of mesangial cells, matrix hypertrophy, visible balloon adhesions, and capillary loop collapse in the kidney. Treatment with prednisone or WHF for 8 weeks significantly ameliorated these morphological alterations.

When examined under an electron microscope, the pathological changes in the kidney in doxorubicin nephropathy rats resembled those of minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS) in humans. In the Con group, no apparent damage was observed, as judged by the intact renal glomeruli (Figure 4). In contrast, in the Adr group, the foot processes tended to be flat and much wider than those in the Con group, and extensive effacement was observed (Figure 4). Thickening of the glomerular basement membrane (GBM) and enlargement of infiltration pores could also be observed. In all the treatment groups, the lesions were significantly attenuated (Figure 4).

WHF significantly represses TRPC6 pathway in rat kidney cortex

Extensive evidence demonstrated that TRPC6 overexpression plays a pivotal role in acquired nephrotic disease. Elevated TRPC6 levels could lead to podocyte dysfunction and proteinuria. To explore the mecha-
A: con: control group; B: Adr: doxorubicin-nephropathy group; C: Pre: prednisone-treated (6.45 mg·kg⁻¹·d⁻¹, 8 weeks in total); D: Wl: low-dose (0.81 g·kg⁻¹·d⁻¹, 8 weeks in total); E: Wm: medium-dose (2.42 g·kg⁻¹·d⁻¹, 8 weeks in total); F: Wh: high-dose (7.26 g·kg⁻¹·d⁻¹, 8 weeks in total).

TRPC6 pathway is partially involved in the protective effect of WHF against doxorubicin-induced nephropathy in rats

A-F: typical Western blot stripes and statistical columns of semi-quantitative Western blot results of TRPC6, STIM1, and Orai1; G-I: statistical columns of real-time RT-PCR results of TRPC6, STIM1, and Orai1. Con: control group; Adr: doxorubicin-nephropathy group; Pre: prednisone-treated (6.45 mg·kg⁻¹·d⁻¹, 8 weeks in total); Wl: low-dose (0.81 g·kg⁻¹·d⁻¹, 8 weeks in total); Wm: medium-dose (2.42 g·kg⁻¹·d⁻¹, 8 weeks in total). Wh: high-dose (7.26 g·kg⁻¹·d⁻¹, 8 weeks in total). TRPC6: transient receptor potential channel subfamily C member 6; WHF: Wenyang Huazhuo Fang; STIM1: stromal interaction molecule 1; Orai1: calcium release-activated calcium channel protein 1; RT-PCR: reverse transcription-polymerase chain reaction. Values are mean ± standard error of mean. *P < 0.01, **P < 0.05 vs Adr; †P < 0.01 vs Con.

Upregulation of TRPC6 and STIM1 protein compared with the Adr group (Figure 5A, 5B, 5D, 5E).

The mRNA levels of TRPC6, STIM1, and Orai1 were also examined. TRPC6, STIM1, and Orai1 mRNA expression in the kidney of doxorubicin-nephropathy rats was highly upregulated compared with those of the Con rats (Figure 5G-5I). Prednisone or WHF treatment markedly inhibited the upregulation of renal TRPC6 and STIM1 mRNA (Figure 5G, 5H). However, the enhancement of Orai1 expres-
sion was significantly reduced by high-dose WHF decoction (Figure 5I). These results suggest that treatment with WHF significantly suppresses the TRPC6 pathway, which is activated in doxorubicin-induced nephropathy in rats.

**DISCUSSION**

The current study demonstrates that the TCM decoction, WHF, can significantly ameliorate proteinuria and improve other biochemical indices in doxorubicin-nephropathy rats, and these effects could be partially related to TRPC6 pathway suppression. Nephrotic syndrome is a series of clinical symptoms, including massive proteinuria, hypoalbuminemia, edema, hyperlipidemia, and lipiduria. Based on response to steroids, the disease can be classified into steroid-responsive and steroid-resistant nephrotic syndrome. According to its histological characteristics, the disease can be categorized into the following types: minimal change nephrotic syndrome, focal segmental glomerulosclerosis, membranoproliferative glomerulonephritis, membranous nephropathy, proliferative glomerulonephritis, mesangial proliferation, and focal and global glomerulosclerosis. Despite rapid development in the field, effective treatment for nephrotic syndrome remains limited. Thus, it is imperative to explore effective and safe therapies for this disease.

WHF was originally developed according to the TCM theory. From a modern pharmacological point of view, the active ingredients in this decoction could exert effects such as anti-inflammation, immunoregulation, lipid regulation, diuresis, anticoagulation, and inhibition of urinary protein excretion. In clinical practice, we have found that WHF decoction potently ameliorates edema and reduces proteinuria. Many animal models have been developed to study the underlying mechanism of nephropathy. In 1988, Orikasa et al. reported that a single intravenous injection of a monoclonal antibody induced massive proteinuria in rats. In the present study, we utilized a doxorubicin-nephropathy rat model, as previously described. Doxorubicin exerts direct toxic damage to the glomerular filtration barrier, including the glomerular endothelial cells, GBM, and podocytes. Furthermore, it was demonstrated that doxorubicin could induce direct acute cellular changes, including alterations in DNA structure (intercalation, crosslinking, or binding), inhibition of topoisomerase II, free radical generation that causes DNA damage and lipid peroxidation, direct cell membrane effects, necrosis, apoptosis, and promotion of senescence-like growth arrest. Doxorubicin-induced glomerulopathy is characterized by the enlargement of glomerular endothelial cell pore size, reduction in glomerular charge selectivity, thickening of GBM, and fusion of podocyte cell foot processes. In China, WHF has been used in TCM for a long time and is widely used in the treatment of nephrotic syndrome.

The renal glomerular filtration barrier is an important structure, and the integrity of this barrier determines the permeability for plasma protein. The glomerular filtration barrier comprises 3 layers, which include a fenestrated endothelium, GBM, podocyte, and slit diaphragm. Physiologically, proteins with sizes that are equal to or larger than that of albumin (69 kDa) are filtered through the slit diaphragms. In nephrotic condition, the glomeruli undergo morphological changes, with adjacent podocytes fusing and flattening, thus losing their foot-like morphology. Damage to the structure and function of glomerular filtration barrier leads to protein leakage into the urine. Based on recent insights into the molecular pathology of podocyte injury, disruption of the complex structure of slit diaphragm has been identified as the major reason for foot-process fusion.

The slit diaphragm complex is a dispensable part that constitutes the glomerular filtration barrier. Moreover, this complex plays a vital role in the cell signaling pathway. Many molecules assemble to form the glomerular slit diaphragm complex, including nephrin, TRPC6, CD2-associated protein, zonula occludens-1, P-cadherin, and podocin, some of which are critical for its integrity. Extensive evidence has shown that TRPC6 is found in podocyte foot processes, and colocalizes with nephrin and podocin along the slit diaphragm. TRPC6 is a member of the large transient receptor potential superfamily of non-selective cation channels that regulates calcium influx. It was found that TRPC6 expression in the podocytes and glomerulus is enhanced in nephropathy.

Store-operated calcium entry (SOCE) is mediated via the activation of specific plasma membrane channels, termed as store-operated calcium (SOC) channels. STIM and Orai proteins are important components of SOCE. STIM1 is a Ca²⁺ sensor in the endoplasmic reticulum, and Orai proteins are pore-forming subunits in the plasma membrane. Orai1 oligomerizes and translocates to the plasma membrane during Ca²⁺ store depletion, thereby triggering Ca²⁺ entry via Orai1. STIM proteins sense the depletion of Ca²⁺ from the endoplasmic reticulum, oligomerize, translocate to junctions adjacent to the plasma membrane, organize Orai or TRPC channels into clusters, and open these channels to allow SOC entry. STIM proteins have been identified as the molecular link between endoplasmic reticulum Ca²⁺ store depletion and SOCE, and calcium release-activated channel activation in the plasma membrane; Orai proteins comprise the calcium release-activated channel pore-forming subunit.

In conclusion, in this study, we found that WHF significantly inhibited weight loss, hypoalbuminemia, proteinuria, and dyslipidemia induced by doxorubicin injection in rats. Microscopic observation revealed that the pathological changes in doxorubicin-nephropathy...
rats were ameliorated by WHF treatment. Ultramicrostructural observations indicated that both foot process effacement and flattening were attenuated by WHF treatment. Furthermore, WHF significantly decreased TRPC6, STIM1, and Orai1 upregulation induced by doxorubicin in rats. Notably, at high dose (40 mL·kg⁻¹·d⁻¹), WHF decoction exerted better therapeutic effects than prednisone treatment did. These findings show that WHF provides a promising TCM treatment for nephrotic syndrome, and emphasize TRPC6 pathway as a target for research in this field.

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