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EXPERIMENTAL STUDY

Qiguiyishen decoction reduced the accumulation of extracellular matrix in the kidneys of rats with adriamycin-induced nephropathy

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Abstract

OBJECTIVE: To investigate the effect of Qiguiyishen decoction (QGYS) on the severity of nephropathy.

METHODS: Twenty-four rats were randomly divided into four groups (Ⅰ, Ⅱ, Ⅲ, Ⅳ) according to the random number table. Group Ⅰ as control group did not establish nephropathy model. Groups Ⅱ, Ⅲ, and Ⅳ were intravenously administered Adriamycin (7.5 mg/kg) through the tail vein to establish nephropathy model. QGYS was prepared with the extracts of Huangqi (*Radix Astragali Mongolici*), Danggui (*Radix Angelicae Sinensis*), Niuxi (*Radix Achyranthis Bidentatae*), and Chuanxiong (*Rhizoma Chuanxiong*). Group Ⅳ was administered QGYS (2 mL·kg^{-1}·d^{-1}), group Ⅲ was administered benazepril (10 mL·kg^{-1}·d^{-1}), and group Ⅰ, Ⅱ was administered water (2 mL·kg^{-1}·d^{-1}) once daily for eight weeks.

RESULTS: QGYS reduced the excretion of urinary protein and N-acetyl-β-D-glucosaminidase and alleviated the accumulation of extracellular matrix (ECM) in renal tissue. Additionally, QGYS effectively regulated the levels of transforming growth factor, tissue inhibitor of matrix metalloproteinase, and matrix metalloproteinases in the kidney of the rats.

CONCLUSION: QGYS may reduce the accumulation of ECM in the kidneys of rats with Adriamycin-induced nephropathy.

Key words: Qiguiyishen decoction; Extracellular matrix; Doxorubicin; Nephrosis

INTRODUCTION

Most chronic kidney diseases (CKD) are types of chronic glomerulonephritis (CGN). Chinese herbs in our decoction have been used to treat CGN for more than one century. We used a decoction derived from Huangqi (*Radix Astragali Mongolici*), Danggui (*Radix Angelicae Sinensis*), Niuxi (*Radix Achyranthis Bidentatae*), and Chuanxiong (*Rhizoma Chuanxiong*) to treat CGN. Nephropathy is a kidney disease that includes glomerulosclerosis (GS) and renal interstitial fibrosis (RIF). High urine protein is the clinical characteristic of nephropathy and the accumulation of extracellular

matrix (ECM) is the pathological feature. CKDs can be induced in rats by the injection of Adriamycin (ADR).[1] The abnormal accumulation and deposition of ECM is the main cause of renal scarring and renal fibrosis.[2] Matrix metalloproteinases (MMP) are a specific group of zinc-dependent protein hydrolysis enzymes that can break down ECM. In renal tissue, tissue inhibitors of matrix metalloproteinases (TIMPs) are the specific inhibitors of MMPs. Recent studies indicate that MMPs, especially MMP-2 and MMP-9, play a role in the regulation of kidney ECM metabolism.[3,4] In our study, we studied the treatment effect of QGYS on Adriamycin-induced nephropathy.

MATERIALS AND METHODS

Drugs and chemicals

Drugs and plant material used were: Adriamycin (doxorubicin hydrochloride, Adrim; HaiZheng Pharmaceuticals, Zhejiang, China), Huangqi (*Radix Astragali Mongolici*), Danggui (*Radix Angelicae Sinensis*), Niuxi (*Radix Achyranthis Bidentatae*), and Chuanxiong (*Rhizoma Chuanxiong*) (Suzhou Tianling Pharmaceutical Co., Ltd., Suzhou, China), and benazepril (Lotensin, Novartis, Beijing, China). All other chemicals and solvents used in the present experiment were of analytical grade.

The decoction was prepared with the crude herbs Huangqi (*Radix Astragali Mongolici*) 600 g, Danggui (*Radix Angelicae Sinensis*) 200 g, Niuxi (*Radix Achyranthis Bidentatae*) 200 g, and Chuanxiong (*Rhizoma Chuanxiong*) 200 g. Herbs were soaked in 2000 mL distilled water for 30 min, and then boiled it for 30 min. The decoction was filtered and condensed to 1000 mL. Professor Jiade Shao, Department of Natural Medicines, the First Affiliate Hospital of Nanjing Traditional Chinese Medicine University School of Pharmaceutical Sciences, identified the botanical origin of the four herbs, and supervised the preparation of the decoction and quality control of QGYS.

Experimental animals and treatment

Animals were supplied by the Shanghai Laboratory Animal Center [certificate of quality SCXK (Shanghai) 2007-0005]. Male SD albino rats (140 ± 10) g were housed under standard temperature (23℃ ± 1℃), relative humidity (55% ± 10%), and 12 h light/12 h dark cycle with access to food and water ad libitum. The study was approved by the Animal Ethics Committee of the First Affiliated Hospital of Soochow University. Rats were randomly assigned to group Ⅰ (sham operation), group Ⅱ, group Ⅲ, or group Ⅳ according to the random numble table. Groups Ⅱ, Ⅲ, and Ⅳ were intravenously administered Adriamycin (7.5 mg/kg) through the tail vein to establish the nephropathy model. One week later, group Ⅳ rats were treated with QGYS, group Ⅲ was given benazepril, and group Ⅱ was administered the same volume of water once daily for eight weeks. Benazepril and QGYS were given at the human equivalent doses of 10 $mL \cdot kg^{-1} \cdot d^{-1}$ (equal to 10 $mL \cdot kg^{-1} \cdot d^{-1}$ in humans) and 1 $g \cdot kg^{-1} \cdot d^{-1}$ (equal to 2 $mL \cdot kg^{-1} \cdot d^{-1}$ in humans), respectively. The dose of QGYS was selected because it was shown to be the minimal dose that exhibited a maximal inhibiting effect on TGF-β_1 mRNA expression in Adriamycin-induced nephropathy rats in our preliminary experiment using 2, 1, and 0.5 g/kg of QGYS.

Testing of urine and blood

Before sacrifice, the rats were placed in metabolic cages on day 7, 28, 42, and 56 for 24-h urine collection. Rats remained on a normal diet with an unrestricted fluid intake. At the end of the experiment, the volume of urine was measured. Individually collected urine (5 mL) was centrifuged at 5000 ×*g* for 5 min. The supernatant was collected and stored at - 80℃. Blood (3 mL) was obtained from each rat via the abdominal aorta on day 56 and then centrifuged at 5000 ×*g* at room temperature for 10 min. The serum was collected and stored at - 80℃ for the detection of serum albumin (SAL), plasma lipids, serum creatinine (SCr), and blood urea nitrogen (BUN). These indicators were examined by an automatic biochemistry analyzer (OLYMPUS AU- 2700, Tokyo, Japan). Radioimmunoassay was used for the detection of urine NAG concentration. The 24-h urine protein excretion was determined by urinary protein and 24-h urine volume.

Preparation of kidney samples

In every 2-week experimental period, the body weight of rats was recorded. At the end of the 8 weeks, animals were sacrificed. Kidneys were harvested and weighed after removal of the envelopes. The kidney/body weight ratio (K/B) was then determined.

Immunohistochemistry analysis

Renal tissue samples were fixed in 10% neutral formaldehyde (Bio-no company, Suzhou, China), dehydrated with ethanol (Bio-no company, Suzhou, China), and embedded in paraffin. Serial sections (4 μm) were collected sequentially on glass slides (Yao hua glass instrument company, Yanchen, China). Paraffin was removed from the sections with xylene and sections were rehydrated in graded ethanol. To retrieve antigenicity from formalin fixation, we incubated the sections for 10 min in 10 mmol/L sodium citrate buffer using a microwave oven (Midea company, Fushan, China). Endogenous peroxidase activity was blocked by further pretreatment with 3% hydrogen peroxide (H_2O_2) (Baiyun pharmaceutical Co., Ltd., Nanchang, China) and methanol. Finally, the sections were incubated with primary monoclonal antibodies against MMP-9, TIMP-1, fibronectin (FN), and collagen Ⅳ (Col-Ⅳ) (Abcam compamy, Combridge, USA) overnight at 4℃. Sections were washed thoroughly in phosphate-buffered saline (PBS) and incubated with rabbit anti-mouse biotinylated second antibody immunoglobulin for 30 min, followed by the avidin-biotin peroxi-