Food and Chemical Toxicology 48 (2010) 522–529

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Wen-pi-tang-Hab-Wu-ling-san reduces ureteral obstructive renal fibrosis by the reduction of oxidative stress, inflammation, and TGF-β/Smad2/3 signaling

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ARTICLE INFO

Article history:
Received 1 July 2009
Accepted 4 November 2009

Keywords:
Fibrosis
Unilateral ureteral obstruction
TGF-β
Smad2/3
Oxidative stress
Inflammation

ABSTRACT

Kidney fibrosis results in chronic renal disease. The current treatment of chronic renal diseases is limited to angiotensin converting enzyme inhibitors and angiotensin receptor blockers. Recently, we found that Wen-pi-tang-Hab-Wu-ling-san (WHW) extract, which has been used to treat renal diseases in herbal medicine for a long time, plays anti-fibrogenic. Here, we investigated the role of WHW in the kidney fibrosis induced by unilateral ureteral obstruction (UUO) in mice. C57BL/6 male mice were subjected to UUO on day 0 and then administered with either WHW (2, 10, or 50 mg/kg of body weight) or vehicle orally from 1 day after UUO to finish the experiment. WHW-administration significantly mitigated the UUO-induced kidney fibrotic changes including tubular atrophy and dilatation, collagen accumulation, expansion of interstitial space and leukocyte infiltration. WHW prevented the increases of oxidative stress by the prevention of UUO-induced decreases of catalase, copper–zinc superoxide dismutase (CuZn-SOD) and manganese superoxide dismutase (MnSOD), resulting in reduced production of oxidative stress. Furthermore, WHW reduced transforming growth factor-β (TGF-β) expression and phosphorylation of Smad2/3 stimulated by UUO. In conclusion, WHW prevented kidney fibrosis following UUO by the inhibition of inflammation, oxidative stress and TGF-β/Smad2/3 signaling pathway.

1. Introduction

The incidence of chronic renal diseases is increasing worldwidely and currently. Although the therapeutics is greatly required, the current treatment of chronic renal disease is limited to angiotensin converting enzyme inhibitors and angiotensin II receptor blockers. In herbal medicine Wen-pi-tang-Hab-Wu-ling-san (WHW) has been used to treat renal diseases for a long time. It suggests that the herbal prescription may be used to develop the therapeutics of chronic renal diseases. However, the lack of studies to clarify its effect and mechanism limits its use widely.

Kidney fibrosis is a common morphological feature of progressive chronic renal diseases including ureteral obstructive nephropathy, diabetic nephropathy, and acute kidney injury (Becker and Hewitson, 2000; Klahr and Morrissey, 2002; Park et al., 2003). The progression of fibrosis is caused by oxidative stress, inflammatory responses, and activation of transforming growth factor (TGF)-β/Smad2/3 signaling (Klahr and Morrissey, 1998, 2002; Forbes et al., 2000; Basile et al., 2001; Seok et al., 2008).

Recently, we found that WHW protected kidney against ischemia and reperfusion (I/R) injury in mice (Seok et al., 2007) and its effect is associated with anti-oxidant. In addition, we found that WHW inhibited the conversion of kidney tubular epithelial cells to mesenchymal cells, a major cause and consequence of kidney fibrosis, induced by TGF-β treatment (Lee et al., 2007). Therefore we hypothesized that WHW prevents the progression of chronic renal disease resulting from kidney fibrosis. To access the hypothesis, we investigated whether WHW prevent the kidney fibrosis induced by unilateral ureteral obstruction (UUO) in mice and the mechanism is associated with inflammatory responses, oxidative stress and TGF-β/Smad2/3 signaling pathway. This study provides WHW effect and acting mechanism in renal diseases.

Abbreviations: WHW, Wen-pi-tang-Hab-Wu-ling-san; UUO, unilateral ureteral obstruction; α-SMA, α-smooth muscle actin; CuZnSOD, copper–zinc superoxide dismutase; MnSOD, manganese superoxide dismutase; TGF-β, transforming growth factor-β; I/R, ischemia/reperfusion; DHE, dihydroethidium; ROS, reactive oxygen species; GFR, glomerular filtration rate; ECM, extracellular matrix; EMT, epithelial to mesenchymal cell transition.

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doi:10.1016/j.fct.2009.11.006

2. Materials and methods

2.1. Preparation of WHW extract

WHW extract was prepared as described previously (Seok et al., 2007, 2008). Briefly, WHW extract was prepared using the following 14 herbs: 150 g of Codonopsis pilosulae radix (*Codonopsis pilosula* FR. NANNF), 150 g of Salviae miltiorrhizae radix (*Salvia miltiorrhiza* BGE), 100 g of Pinelliae rhizome (*Pinellia ternata* THUNB. BREIT.), 60 g of Coptis rhizome (*Coptis chinensis* FRANCH), 160 g of Epimedii herba (*Epimedium koreanum* NAKAI), 100 g of Rhei radix et rhizoma (*Rheum palmatum* L.), 100 g of Perillae folium (*Perilla frutescens* L. BRITT.), 50 g of Glycyrrhizae radix (*Glycyrrhiza uralensis* FISCH), 300 g of Artemisiae capillaris herba (*Artemisia capillaris* THUNB.), 200 g of Alismatis rhizome (*Alisma plantago-aquatica var. orientale* SAMUELS), 80 g of Poria (*Poria cocos* SCHW.), 80 g of Atractylodis macrocephalae rhizome (*Atractylodes macrocephala* KOIDZ.), 80 g of Polyporus (*Polyporus umbellatus* PERS. FRIES), and 40 g of Cinnamomi ramulus (*Cinnamomum cassia* PRESL). Plants were purchased from Medicinal Materials Co. (Youngcheon Province) and authenticated by Prof. Young-Ki Park, a medical botanist in the Department of Herbalogy, College of Oriental Medicine, Dongguk University (DUCOM). The voucher specimens (OB05-1) have been deposited in the Herbarium of DUCOM. WHW was extracted from a crude herb mixture (1700 g) by boiling in water for 5 h followed by filtering through a two-layer mesh and concentration in a boiling water bath to obtain residues (yields of 18.5%). These extracts were stored at 4 °C before use. For administration of WHW, the extract was suspended in 0.9% NaCl. We previously confirmed the phytochemical characterization of Wen-pi-tang-Hab-Wu-ling-san (WHW) using thin-layer chromatography (TLC) and fingerprint high performance liquid chromatography (HPLC) analysis (Lee et al., 2007). Rosmarinic acid which had the anti-oxidant effect (Gao et al., 2005) was identified a major chemical of the WHW extract (Lee et al., 2007).

2.2. Animal preparation

Experiments were performed in age-matched (8 week old) C57BL/6 male mice (weighed 20–25 g). All studies were conducted in accordance with the Animal Care and Use Committee guidelines of Kyungpook National University. To induce UUO, the left kidneys were exposed through flank incisions and a kidney ureter was tied completely using 6/0 nylon under anesthetization with pentobarbital sodium (60 mg/kg body weight). Some mice had either WHW extract (2, 10 or 50 mg/kg BW) or 0.9% NaCl (vehicle) administered orally beginning at 1 day for 6 days after the onset of procedures. Each experimental animal group consisted of 4–6 mice.

2.3. Histology

Kidneys were perfusion-fixed with PLP (2% paraformaldehyde, 75 mM L-lysine, 10 mM sodium periodate; Sigma, St. Louis, MO) fixative as described previously (Park et al., 2001; Kim et al., 2009a). Fixed kidneys were embedded in paraffin and cut into 2 μm sections using a microtome. Sections were stained with periodic acid Schiff (PAS) or Masson trichrome staining following standard protocols.

2.4. Histological score

Five fields (0.1 mm^2/field) per kidney were used. The collagen-positive areas were analyzed using LabWorks Image Acquisition and Analysis Software (Ultra-Violet Products Ltd., Cambridge, UK).

2.5. Dihydroethidium staining

To detect the presence of superoxide anions by dihydroethidium (DHE; Sigma) staining (Djamali, 2007), kidney sections prepared frozen-fixed by embedding in an OCT compound and sectioned to 5 μm thickness. Sections were incubated in 1 mM DHE for 1 h at 37 °C. The density of DHE positive signals was analyzed using the LabWorks program (Ultra-Violet Products Ltd.). Five fields (0.1 mm^2/field) per kidney ($n = 3$) were used for statistical analysis.

2.6. Immunoblot analysis

Protein samples were prepared by homogenizing kidneys in a Dounce homogenizer as described previously (Park et al., 2001; Jang et al., 2008). The resulting homogenate was then prepared for Western blot analysis as described previously (Park et al., 2001). Immunoblot analyses were performed using α-SMA (1:5000; Sigma), catalase (1:10,000; Fitzgerald, Concord, MA), copper–zinc superoxide dismutase (CuZnSOD; 1:2000; Chemicon, Temecula, CA), manganese superoxide dismutase (MnSOD; 1:2000; Calbiochem, San Diego, CA), Ly6G (1:2000; eBioscience, San Diego, CA), phospho-Smad2 (1:2000; Cell signaling, Beverly, MA), phospho-Smad3 (1:1000; Cell signaling), myeloperoxidase (MPO; 1:2000; AbFRON-

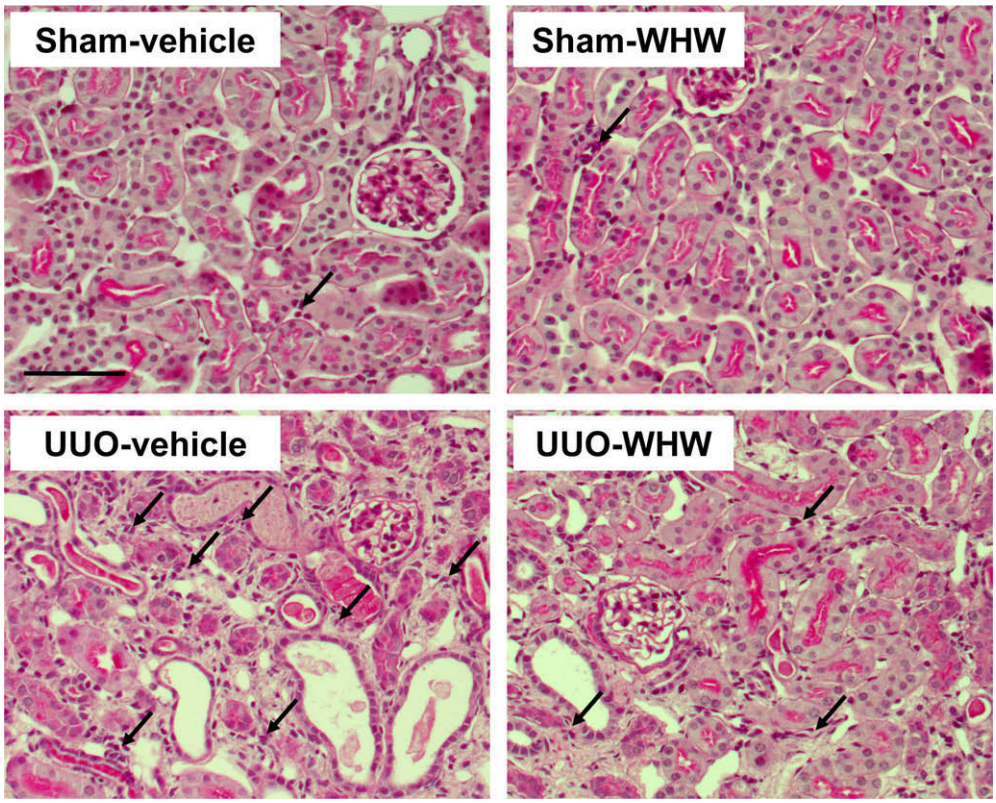

Fig. 1. Morphology of kidneys 7 days after unilateral ureteral obstruction (UUO). C57BL/6 mice were subjected to either UUO or sham operation on day 0. Oral administration with either 10 mg/kg BW of WHW or vehicle began daily at day 1 after UUO for 6 days. On day 7, kidneys were harvested. Kidney sections were prepared in 2 μm slices and stained with periodic acid Schiff (PAS). Bar indicates 50 μm. Arrow indicates interstitial cell.