Inhibition of the Tubular Epithelial-to-Mesenchymal Transition in vivo and in vitro by the Uremic Clearance Granule (尿毒清颗粒)

LU Zhao-yu (卢钊宇)，LIU Shu-wen (刘述文)\(^2\), XIE Yuan-sheng (谢院生)\(^1\), CUI Shao-yuan (崔少远)\(^1\), LIU Xu-sheng (刘旭生)\(^2\), GENG Wen-jia (耿文佳)\(^1\), HU Xiao (呼啸)\(^1\), JI Jia-yao (季佳瑶)\(^1\), and CHEN Xiang-mei (陈香美)\(^1\)

ABSTRACT  Objective: To investigate the effect of the Uremic Clearance Granule (UCG, 尿毒清颗粒), a Chinese patent medicine, on tubular epithelial-to-mesenchymal transition (EMT) in a unilateral ureteral obstruction (UUO) model in vivo and transforming growth factor (TGF)-β1-induced EMT of HK-2 cells in vitro. Methods: In vivo study, 50 Sprague Dawley rats were divided into three groups: a sham operation group (n=10), a UUO group (n=20), and a UUO with UCG treatment group (n=20). The UCG was given at a dose of 4.5 g/kg body weight per day by gavage after surgery. In vitro study, HK-2 cells were cultured in 10% fetal bovine serum (FBS), 10% healthy rat serum, 10% FBS and TGF-β1 (10 ng/mL), 10% healthy rat serum and TGF-β1, or 10% rat serum containing the uremic clearance granule and TGF-β1. The expression of the epithelial marker E-cadherin and the mesenchymal markers vimentin and α-smooth muscle actin (α-SMA) in kidney tissues and HK-2 cells were investigated by Western blot analysis and immunofluorescence staining. Results: The rats of the UG group showed obvious tubulointerstitial fibrosis, compared with the sham operation group rats. Tubulointerstitial fibrosis score was reduced by 17.5% ± 1.1% at day 7 and by 20.0% ± 1.2% at day 14 in the UCG-treated group, compared with the UUO group. The UCG could maintain the epithelial morphology of HK-2 cells in vivo and in vitro. This occurred partially through a reduction of vimentin expression and an increase of E-cadherin expression. Conclusion: These results suggest that the UCG prevents tubular EMT and may be a promising agent for treating tubulointerstitial fibrosis.

KEYWORDS  Uremic Clearance Granule, epithelial-to-mesenchymal transition, tubulointerstitial fibrosis

The severity of tubulointerstitial fibrosis correlates well with the risk for progression to renal failure.\(^1\) Recent studies have demonstrated that a critical step in the pathogenesis of tubulointerstitial fibrosis is the epithelial-to-mesenchymal transition (EMT).\(^2,3\) Whereby renal tubular epithelial cells change phenotype and function into myofibroblasts, which finally induce the excess deposits of extracellular material. Therefore, the EMT in the kidney should be of significant interest as a therapeutic target, and in this regard, it is quite important to prevent tubular EMT during tubulointerstitial fibrosis therapy. Chinese medicine has a rich history of treating chronic kidney diseases over thousands of years, and some herbal medicines and extracts have been demonstrated to contain anti-renal fibrosis properties in vivo\(^4\) and in vitro.\(^5\) Although specific therapies for preventing the progression of chronic kidney diseases are still lacking, seeking drug candidates or herbal compounds that can effectively prevent or inhibit EMT would provide a new strategy for treating chronic kidney disease.

The Uremic Clearance Granule (UCG, 尿毒清颗粒), a Chinese patent medicine, includes Radix et rhizoma rhei, Radix astragali, Radix salviae miltiorrhizae, Radix sophorae flavescentis, Rhizoma
atractylodis macrocephalae, and Radix polygoni multiflori. It has been widely used for treating chronic kidney diseases in China. The clinical efficacy of the UCG has been established,\(^6\),\(^7\) it has been proved to be able to reduce serum creatinine and urea nitrogen, improve renal function. However, its exact mechanism of action is still uncertain. Furthermore, it is unclear whether the UCG has protective effects against tubular EMT.

The unilateral ureteral obstruction (UUO) animal model\(^8\) and the cell model induced by transforming growth factor (TGF)-\(\beta\) 1 in which tubular epithelial cells undergo phenotypic conversion\(^9\) are classic in vivo and in vitro EMT models. In this study, we investigated the effect of treatment with the UCG on EMT in vivo using the UUO model and in vitro using TGF-\(\beta\) 1-induced EMT of HK-2 cells, to elucidate the mechanism by which the UCG inhibits tubulointerstitial fibrosis. Given that the UCG is a complex combination of Chinese herbs, it was difficult to use it directly in an in vitro experimental study. We used the serologic pharmacology method, which has been widely used in Chinese herbal studies,\(^10\),\(^11\) to obtain rat serum containing the UCG composition and investigated whether this serum could prevent or inhibit EMT in vitro. The results suggest that the UCG inhibits tubular EMT and may be a promising agent for treating tubulointerstitial fibrosis.

**METHODS**

**Animals, Cells, Antibodies, and Experimental Drugs**

Fifty specific pathogen free male Sprague Dawley (SD) rats (age, 3 months) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were housed in our animal facility under pathogen-free conditions and were fed a standard laboratory diet, with free access to water. The temperature was maintained at 18–22\(\,\)\(\circ\)C with a 12-h light/dark cycle. All animal procedures complied with the government-published recommendations for the use of laboratory animals.

The human kidney proximal tubular cell line (HK-2, ATCC, Manassas, VA, USA) was cultured in Dulbecco's Modified Eagle Medium (DMEM)/F12 (Invitrogen, Carlsbad, CA, USA) containing 2.50 g/L hydroxyethyl piperazine ethanesulfonic acid (Sigma, St. Louis, MO, USA), 1.80 g/L sodium bicarbonate (Sigma, St. Louis, MO, USA), 100 U/mL penicillin, 100 U/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), at 37\(\,\)\(\circ\)C in 5% CO\(_2\).

The anti-E-cadherin antibody was procured from Abcam (Cambridge, UK), antibodies against \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA) and vimentin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti-\(\beta\)-actin antibody was obtained from Sigma. Fluorescein isocyanate (FITC)-conjugated anti-mouse IgG and cy3-conjugated anti-rabbit IgG were purchased from Jackson ImmunoResearch (West Grove, PA, USA).

**Composition of UCG and Preparation of the Herbal Extract**

The UCG, consists of a defined mixture of 10 herbs as follows: Radix et Rhizoma Rhei, Radix Astragali, Cortex Mori, Radix Salviae Miltiorrhizae, Radix Sophorae Flavescentis, Radix Paeoniae Alba, Rhizoma Atractylodis Macrocephalae, Poria, Radix Polygoni Multiflori Preparata and Herba Plantaginis. The herbs were processed and extracted under the national drug supervision and administration drug standards [WS3-229(Z-033)-2000(Z)], with state drug approval document No. Z20073256. Each extract was monitored for the absence of contaminants (heavy metals, pesticides, and mycotoxins) prior to formulation.

**Establishing the UUO Model**

On the day of the operation, 50 SD rats were randomly divided into three groups: a sham operation group (n=10), a UUO group (n=20), and a UUO with UCG treatment group (UUO+UCG, n=20). All rats were anesthetized with 2% pentobarbital sodium (30 mg/kg body weight, intraperitoneally). The UUO model was developed using an established procedure.\(^12\) Briefly, a complete ureteral obstruction was performed by double ligating the left ureter using 4-0 silk after a left lateral abdominal incision. The UCG was given at a dose of 4.5 g/kg body weight per day (about 10 times the adult human dose) by gavage after surgery. The rats in the sham operation group and UUO group received the same volume of saline solution.

Rats were sacrificed at 7 and 14 days (n=10 per group) after surgery, and the kidneys were decapsulated and divided into several parts. One