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Rutin ameliorates kidney interstitial fibrosis in rats with obstructive nephropathy

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ABSTRACT

Rutin reportedly conveys many beneficial effects, including renoprotection; however, it has not yet been demonstrated to have a renoprotective effect against obstructive nephropathy. The present study is the first to show a protective effect of rutin against obstructive renal injury induced by unilateral ureteral obstruction (UUO). A total of 24 male Wistar rats were randomly divided into four groups of six rats each, including vehicle- or rutin-treated sham operated groups, and vehicle- or rutin-treated UUO groups. Rats received daily oral gavage of rutin (100 mg/kg) for 2 weeks. All rats were euthanized on postoperative day 14. Histological findings showed that rutin administration significantly reduced renal interstitial injury and suppressed interstitial collagen deposits in UUO rats. Moreover, rutin decreased macrophage infiltration, proinflammatory cytokine expression and phosphorylation of nuclear factor-κB p65. Furthermore, rutin inhibited extracellular matrix accumulation by reducing expression of type I/III collagen and fibronectin. Rutin also prevented the epithelial-mesenchymal transition processes of renal tubular cells by decreasing α-smooth muscle actin expression and retaining E-cadherin expression. These effects of rutin were in parallel with the reductions in Smad3 activity and pivotal to the fibrogenic potential of TGF-β1. Taken together, the renoprotective effects of rutin in obstructive nephropathy were likely due to anti-inflammatory effects and inhibition of TGF-β1/Smad3 signaling.

1. Introduction

Congenital obstructive nephropathy is the most recognizable cause of chronic kidney disease in newborns and children [1]. Renal tubulointerstitial fibrosis, a common hallmark of progressive chronic renal diseases, including obstructive nephropathy, is characterized by inflammatory cell infiltration, myofibroblast proliferation, and accumulation of extracellular matrix (ECM) [2]. Generation of myofibroblasts is considered a key process in tubulointerstitial fibrosis, which accounts for the accumulation of ECM under diseased conditions. Epithelial to mesenchymal transition (EMT), characterized by loss of epithelial proteins (E-cadherin, zonula occludens-1, etc.) and acquisition of new mesenchymal markers (α-smooth muscle actin (α-SMA), vimentin, etc.), is regarded as a pivotal pathway in matrix-producing myofibroblast generation [3]. Of the numerous growth factors involved in the pathophysiology of renal fibrosis, transforming growth factor β1 (TGF-β1) is considered the crucial mediator of initiation and progression of interstitial fibrosis. Activation of TGF-β1 and its downstream signaling protein Smad3 (mothers against decapentaplegic homolog 3) plays important roles in both EMT and consequent ECM accumulation [4,5]. Furthermore, recruitment of inflammatory cells, secretion of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), as well as activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) also contribute to the progression of tubulointerstitial fibrosis [6]. Although considerable advances have been made in the prevention of deleterious effects in obstructive nephropathy, strategies to protect patients from the damage of obstructive nephropathy remain urgent.

Rutin is a common polyphenolic bioflavonoid widely found in many foods and traditional medicines that has a broad spectrum of pharmacological properties, including anti-oxidative, anti-inflammatory, antiallergic, anticarcinogenic, antimicrobial, antiviral,

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and cytoprotective activities [7,8]. Besides, recent studies have indicated the potential use of rutin against hepatic fibrosis caused by bile duct ligation [9]. Several studies also demonstrate that rutin has significant beneficial kidney protective effects in ischemia/reperfusion renal injury [10,11], drug-induced renal injury [12–14], and diabetic nephropathy [15–17].

However, the effectiveness of rutin against tubulointerstitial fibrosis, inflammation, and renal tubular EMT in obstructive nephropathy has not yet been explored. Therefore, the present study was conducted to examine the protective effects of rutin in unilateral ureteral obstruction (UUO)-inflicted rats and to identify possible underlying mechanisms.

2. Materials and methods

2.1. Reagents and antibodies

Rutin hydrate (≥94%) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Antibodies against collagen I, collagen III, fibronectin, α-SMA, E-cadherin, TGF-β1, TNF-α, IL-1β, and CD68 were purchased from Abcam (Cambridge, MA, USA). Antibodies against p-NF-κB and p-Smad3 were purchased from Cell Signaling Technology (Danvers, MA, USA). Other reagents and antibodies were purchased from ZSGB-BIO (Beijing, China).

2.2. UUO model and treatment

The experimental protocol was approved by the Ethics Review Committees for Animal Experimentation of Southern Medical University (Guangdong, China). Male Wistar rats, weighing 200–250 g, were purchased from Southern Medical University Laboratory Animal Center (Guangzhou, China) and housed in an air-conditioned room at 25 °C under a 12/12-h light/dark cycle with access to food and water ad libitum. All animals received humane care in compliance with the university's guidelines. After 1 week of acclimatization, the rats were randomly divided into four groups ($n = 6$/group): sham and UUO groups with vehicle treatment, and sham and UUO groups with rutin treatment. UUO was performed as previously described [18]. Briefly, after intraperitoneal anesthesia with pentobarbital sodium (50 mg/kg), the abdominal cavity was exposed via a midline incision. The left proximal ureter was ligated at two locations with 4-0 silk sutures and cut between the knots. Rats subjected to sham surgery underwent the same procedure, but without ureter ligation. After recovering from general anesthesia, each rat received oral gavage of rutin (100 mg/kg) dissolved in 1% carboxymethylcellulose-Na (CMC-Na) buffer daily for 13 days. The dosage and administration of rutin were assessed based on our preliminary tests and relevant studies [16,17,19]. CMC-Na buffer (1%) was used as a vehicle. Two weeks after surgery, the rats were euthanized and the left kidneys were harvested. One part of the left kidney was fixed in 4% paraformaldehyde for histological examination and the remaining tissue was rapidly frozen in liquid nitrogen and stored at −80 °C for protein extraction.

2.3. Histopathological examination

The kidneys were transversely cut into 4-μm sections. Deparaffinized sections of kidney tissues were stained with hematoxylin and eosin (H&E) and Masson's trichrome for morphological studies. Renal tubulointerstitial injury was examined by H&E staining and evaluated semiquantitatively, as described previously [20]. Briefly, 10 tubulointerstitial fields at 200× magnification were randomly

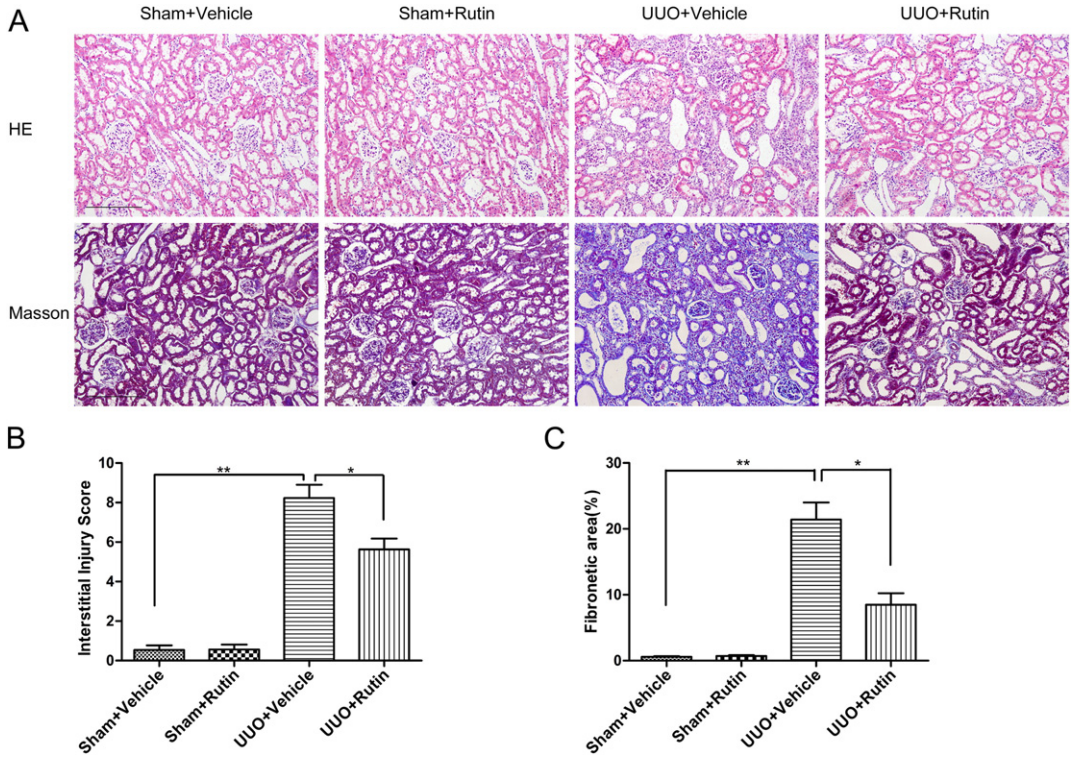

Fig. 1. Rutin attenuates tubulointerstitial injury and fibrosis in the obstructed kidneys 14 days after UUO. (A) Representative images of renal sections stained with H&E and Masson's trichrome (Masson). Scale bar indicates 200 μm. Semiquantitative analysis of interstitial injury score (B) and relative fibrogenic area (%) (C) of the obstructed kidneys in each group. All values are expressed as the mean ± SD ($n = 6$). ** indicates $p < 0.001$ and * indicates $p < 0.05$.