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Arctigenin suppresses renal interstitial fibrosis in a rat model of obstructive nephropathy

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ABSTRACT

Background: Renal tubulointerstitial fibrosis (TIF) is commonly the final result of a variety of progressive injuries and leads to end-stage renal disease. There are few therapeutic agents currently available for retarding the development of renal TIF.

Purpose: The aim of the present study is to evaluate the role of arctigenin (ATG), a lignan component derived from dried burdock (*Arctium lappa* L.) fruits, in protecting the kidney against injury by unilateral ureteral obstruction (UUO) in rats.

Methods: Rats were subjected to UUO and then administered with vehicle, ATG (1 and 3 mg/kg/d), or losartan (20 mg/kg/d) for 11 consecutive days. The renoprotective effects of ATG were evaluated by histological examination and multiple biochemical assays.

Results: Our results suggest that ATG significantly protected the kidney from injury by reducing tubular dilatation, epithelial atrophy, collagen deposition, and tubulointerstitial compartment expansion. ATG administration dramatically decreased macrophage (CD68-positive cell) infiltration. Meanwhile, ATG downregulated the mRNA levels of pro-inflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) and cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ), in the obstructed kidneys. This was associated with decreased activation of nuclear factor κB (NF-κB). ATG attenuated UUO-induced oxidative stress by increasing the activity of renal manganese superoxide dismutase (SOD2), leading to reduced levels of lipid peroxidation. Furthermore, ATG inhibited the epithelial-mesenchymal transition (EMT) of renal tubules by reducing the abundance of transforming growth factor-β1 (TGF-β1) and its type I receptor, suppressing Smad2/3 phosphorylation and nuclear translocation, and up-regulating Smad7 expression. Notably, the efficacy of ATG in renal protection was comparable or even superior to losartan.

Conclusion: ATG could protect the kidney from UUO-induced injury and fibrogenesis by suppressing inflammation, oxidative stress, and tubular EMT, thus supporting the potential role of ATG in renal fibrosis treatment.

Introduction

Chronic kidney disease (CKD) has emerged as a worldwide public health problem with a rapid growth in prevalence (Couser et al., 2011). Progressive tubulointerstitial fibrosis (TIF) is the common pathological presentation of nearly all kinds of CKD leading to end-stage renal failure (Liu, 2006). Although tremendous efforts have been made to prevent or retard the progression of TIF,

Abbreviations: α-SMA, α-smooth muscle actin; ANOVA, analysis of variance; ATG, arctigenin; BCA, bicinchoninic acid; BUN, blood urea nitrogen; CAT, catalase; CKD, chronic kidney disease; Cr, creatinine; DAB, 3, 3′-diaminobenzidine; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; GPx, Glutathione peroxidase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; HYP, hydroxyproline; IFN-γ, interferon-γ; IL-1β, interleukin-1β; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; NF-κB, nuclear factor-κB; PVDF, polyvinylidene difluoride; qPCR, quantitative real-time PCR; SOD1, copper-zinc superoxide dismutase; SOD2, manganese superoxide dismutase; TβR-I, TGF-β type I receptor; TβR-II, TGF-β type II receptor; TBA, thiobarbituric acid; TECs, tubular epithelial cells; TGF-β1, transforming growth factor-β1; TIF, tubulointerstitial fibrosis; TNF-α, tumor necrosis factor-α; UUO, unilateral ureteral obstruction.

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specific drug therapies to delay the progression of TIF toward end-stage renal failure are limited.

CKD can initially manifest as inflammatory responses characterized by infiltration of immune cells, mainly monocytes/macrophages and T lymphocytes, into the glomeruli and tubulointerstitium (Lopez-Novoa and Nieto, 2009). On one hand, these inflammatory cells secrete various pro-inflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ), and monocyte chemoattractant protein-1 (MCP-1), which further contribute to recruitment of circulating inflammatory cells. This creates a malignant positive feedback loop of inflammation (Diamond et al., 1994). On the other hand, activated macrophages produce profibrotic cytokines such as transforming growth factor-β1 (TGF-β1), which have been shown to induce activation of matrix-producing myofibroblasts (Leask and Abraham, 2004). The stimulated myofibroblasts cause accumulation of extracellular matrix (ECM) proteins and lead to complete destruction of renal parenchyma and irreversible renal failure. Renal inflammation—and in particular the accumulation of macrophages in renal interstitium—are a critical element in the mechanism responsible for the initiation and development of renal fibrogenesis. Therefore, inhibiting inflammatory responses may significantly attenuate renal TIF.

Recent studies show that a significant portion of synthetically active myofibroblasts arise from renal tubular epithelial cells (TECs) *via* the epithelial-mesenchymal transition (EMT) in fibrotic kidney diseases (Liu, 2010). The pro-fibrogenic effect of inflammation depends, at least partially, on triggering EMT (Wynn, 2008). Several studies have shown that sustained stimulation of pro-inflammatory cytokines TNF-α or IL-1 can induce EMT in the epithelial cell lines (Takahashi et al., 2010). During EMT, differentiated TECs lose their epithelial characteristics and undergo multiple biochemical changes, which enable them to assume a mesenchymal phenotype. This phenotypic conversion involves the *de novo* synthesis of mesenchymal cytoskeletal biomarkers such as α-smooth muscle actin (α-SMA), fibronectin and vimentin, a down-regulation of epithelial biomarkers such as E-cadherin and zonula occludens-1, and the acquisition of a fibroblastic morphology with a concomitant invasive phenotype. TGF-β1 has been identified as the main inducer of EMT and consequent interstitial ECM production in kidney and other organ systems (Acloque et al., 2009; Pan et al., 2015). The intracellular Smad pathway is important for TGF-β1 to initiate tubular EMT and fibrotic responses (Lan and Chung, 2011). To prevent progression of TIF, the inhibition of tubular EMT mediated by TGF-β1/Smad signaling pathway may be helpful.

Unilateral ureteral obstruction (UUO) is a well-established experimental model used to elucidate pathological mechanisms of chronic obstructive nephropathy (Chevalier et al., 2009). Oxidative stress plays a central role in the progression of renal damage in obstructed nephropathy (Kawada et al., 1999). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of intrinsic antioxidant defense. In UUO kidneys, the superoxide anion ($O_2^{\bullet -}$) and its derivative hydrogen peroxide (H_2O_2) are increased, while the antioxidant enzyme catalase (CAT) and copper-zinc superoxide dismutase (SOD1) mRNA correspondingly decreased (Ricardo et al., 1997). Increased ROS concentrations can cause tubulointerstitial injury by increasing lipid peroxidation, hydrogen peroxides, leukocyte activation, DNA breakdown, protein oxidation, and apoptosis. Furthermore, ROS generated in TECs induce nuclear factor kappa B (NF-κB) activation (Gloire et al., 2006) leading to transcription of some pro-inflammatory mediators such as MCP-1, TNF-α, INF-γ, and IL-1β, infiltration of monocytes/macrophages, proliferation of fibroblasts, and ECM accumulation in the renal interstitium. Initial oxidative stress and inflammation contribute to the progression of renal fibrosis. Thus, antioxidant and/or anti-inflammatory agent have been used to protect the kidney from injury induced by UUO.

Burdock (*Arctium lappa* L.) has been used in traditional and folk medicine in oriental countries for centuries. Various experimental models have shown that arctigenin (ATG) is a main bioactive ingredient derived from dried fruit of *A. lappa*. ATG exhibits anti-inflammatory (Hyam et al., 2013), anti-oxidant (Zhang et al., 2015), and antineoplastic (Awale et al., 2006) properties in various disorders. Our preliminary study demonstrated that oral administration of 95% (v/v) hydroalcoholic extract from *A. lappa* containing a higher amount of ATG and its glycoside Arctiin *versus* aqueous and petroleum ether extracts, exhibited the strongest inhibitory effect on ECM component synthesis in the obstructive kidneys of rats with UUO (Supplementary Fig. S1). Our previous data also showed that ATG could reverse TGF-β1-triggered renal tubular EMT-like phenotypic changes *in vitro* using human proximal tubular epithelial cells (HK-2 cells)—mainly due to the inhibition of TGF-β1-induced up-regulation of MCP-1 (Li et al., 2015). Because the regulatory role of ATG in renal fibrosis *in vivo* has not yet been studied, we studied here a 14-day UUO rat model to assess the anti-fibrotic efficacy of ATG and further delineated the potential molecular mechanisms by which ATG elicits its effects on experimental renal fibrosis.

Materials and methods

Chemicals

Arctigenin (MW: 372.41) was provided by Nanjing Zelang Medical Technology Co. Ltd. (Nanjing, China). The purity of ATG was determined to be > 8% using high performance liquid chromatography (HPLC; Supplementary Fig. S2). Losartan was purchased from Merck Sharp & Dohme Ltd. (Hangzhou, China). Antibodies against the following proteins were used: NF-κB p65 (Signalway Antibody, Pearland, TX, USA); fibronectin (BD Biosciences, San Jose, CA, USA); Smad2/3, phosphorylated Smad2/3 (p-Smad2/3) and vimentin (Cell Signaling Technology, Beverly, MA, USA); E-cadherin, lamin B1, IκBα, TGF-β1, and TGF-β type I (TβR-I) and type II (TβR-II) receptors (Santa Cruz Biotechnology, Santa Cruz, CA, USA); α-SMA, SOD1, manganese superoxide dismutase (SOD2) and Smad7 (Epitomics, Burlingame, CA, USA); β-actin (Bioworld Technology, Minneapolis, MN, USA); collagen type I and CD68 (Boster biological technology, Wuhan, China). Serum creatinine, blood urea nitrogen (BUN), urinary protein, hydroxyproline (HYP), malondialdehyde (MDA), and SOD assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Glutathione peroxidase (GPx), glutathione reductase (GR), CAT, and bicinchoninic acid (BCA) protein assay kits were obtained from Beyotime Institute of Biotechnology (Jiangsu, China). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

Animals

Eight-week-old male Sprague-Dawley rats (180–200 g; certification No: SCXK-Yu-2012-0005) were supplied by the Experimental Animal Center of Third Military Medical University (Chongqing, China). All studies were performed in accordance with the National Institutes of Health guidelines for the *Care and Use of Laboratory Animals* (8th Edition, 2011). The experimental protocols were approved by the Institutional Ethics Committee of Chongqing University of Technology. All rats were housed in an air-conditioned room at 21 ± 2 °C and 50 ± 5% relatively humidity under a 12-h light/dark cycle and had access to standard rodent chow and water *ad libitum* throughout the study period.