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Inhibition of TGF-β1/Smad signal pathway is involved in the effect of *Cordyceps sinensis* against renal fibrosis in 5/6 nephrectomy rats

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ABSTRACT

The present study aimed to investigate the effects of *Cordyceps sinensis* on renal fibrosis and its possible mechanisms. Sprague-Dawley rats were randomly divided into three groups: sham operation (SHAM) group, 5/6 subtotal nephrectomy (SNx) untreated group, and 5/6 subtotal nephrectomy treated with *C. sinensis* (2.0 g/kg d) (CS) group. Rats were studied 12 weeks after the surgery, and the CS group presented with significantly lower proteinuria, and better renal function compared with the SNx group ($p < 0.05$). Pathological study showed that the glomerulosclerosis tubulointerstitial injury score was significantly reduced in the CS group compared with the SNx group. Furthermore, the mRNA expression of TGF-β1, Smad2 and Smad3 and the protein expression of TGF-β1, TβRI, TβRII and p-Smad2/3 were attenuated by the *C. sinensis* treatment. In constrast, the mRNA and protein expression of Smad7 was upregulated. Furthermore, the expression of α-SMA and FSP1 was also significantly attenuated, accompanied by the increasing expression of E-cadherin, suggesting the inhibition of the epithelial–mesenchymal transition (EMT). In conclusion: *C. sinensis* exerted its antifibrotic effect on the SNx rats through the inhibition of the TGF-β1/Smad pathway.

1. Introduction

Chronic kidney disease (CKD) is a global public health problem with a high mortality. Renal fibrosis is the final pathological manifestation of CKD, characterised by tubulointerstitial fibrosis and glomerulosclerosis, and it leads to the deterioration and eventual loss of renal function, independent of the primary cause (Liu, 2006). Although remarkable advances have been made in treating CKD, hundreds of thousands of people develop end stage renal disease (ESRD) every year requiring renal replacement therapy. Therefore, finding new therapies aimed at attenuating renal fibrosis is important.

The degree of renal cortical interstitial fibrosis has been well established to predict the likelihood of progressive renal impairment. Previous studies have demonstrated that the epithelial–mesenchymal transition (EMT), a process in which proximal tubular epithelial cells acquire the phenotypic characteristics of fibroblasts, is a very important step in the pathogenesis of tubulointerstitial fibrosis (Badid et al., 2002; Powell et al., 1999). Furthermore, one mechanism by which the cytokine transforming growth factor-β1 (TGF-β1) has been implicated in the pathogenesis of renal fibrosis is by driving this process (Liu et al., 2012).

The traditional medicine known as Chinese caterpillar fungus (Dongchong Xiacao) is composed of the parasitic fungus *Cordyceps sinensis*, which grows on the larva of the ghost moth *Hepialus armoricanus Oberthur* (Hepiallidae) (Yue et al., 2008). *C. sinensis* has a long history of medical use in China, beginning during the Qing dynasty in A.D. 1694. According to Chinese tradition and the Chinese Pharmacopoeia, *C. sinensis* can "tonify the lung, replenish the kidneys, arrest bleeding, dissolve phlegm, treat chronic coughs, treat spontaneous sweating and restore strength after an illness"(Zhu et al., 1998a, 1998b). More recent studies suggest that Cordyceps may have immunomodulatory (Jordan et al., 2008), pro-apoptotic (Zhang and Wu, 2007), anti-proliferative (Yang et al., 2003) and anti-fibrotic effects (Li et al., 2006; Wang et al., 2007).

We previously demonstrated *in vitro* that Cordyceps antagonises the effects of TGF-β1, inhibiting EMT (Zhang et al., 2012). In addition in an animal model of fibrosis, Cordyceps was able to attenuate renal fibrosis (Zhu et al., 2011), although the *in vivo* mechanism for this effect was unclear. The present study aimed to provide a mechanistic insight into the potential effects of *C. sinensis* in an *in vivo* model of progressive renal fibrosis.

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2. Materials and methods

2.1. Preparation for C. sinensis suspension

Artificially cultured *C. sinensis* was kindly provided by Hangzhou Huadong Pharmaceutical Company (Hangzhou, China) and used at a final concentration of 0.2 g/ml (mixed with double-distilled water).

2.2. Animals and experimental design

Male Sprague-Dawley rats at 6 weeks of age, weighing 200–220 g, were obtained from Shanghai Super-B&K Laboratory Animal Corp. Ltd. (Shanghai, China). All animal experiments were performed with the approval of the Ethical Committee of Southeast University. Animals were maintained under a constant 12-h photoperiod at temperatures between 21 °C and 23 °C, and were allowed free access to food and water.

The remnant kidney model (5/6 nephrectomy) of progressive fibrosis was generated by the removal of the right kidney and selective resection of the upper and lower poles (2/3) of the left kidney under anaesthesia with chloral hydrate. The sham operation consisted of a ventral laparotomy and kidney decapsulation (Eraranta et al., 2012). The 5/6 subtotal nephrectomy rats were randomly divided into two groups: (1) 5/6 subtotal nephrectomy group with no intervention (SNx, $n = 10$) and (2) 5/6 subtotal nephrectomy group treated with *C. sinensis* (2.0 g/kg d by intragastric administration) (CS, $n = 10$). The sham operation rats served as control group (SHAM, $n = 10$). Intragastric administration started at 7 days after operation, and the other two groups were given double-distilled water. The body weights were assessed and the 24-h urine excretion was collected in metabolic cages every two weeks. All animals were sacrificed 12 weeks after the surgery. At the end of the study period and prior to sacrifice, blood samples were taken for biochemical studies, and renal tissue was used for histological assessments.

2.3. Plasma and urine determinations

The concentrations of blood serum urea nitrogen (BUN) and creatinine (Scr) were determined by an automatic analyzer (Hitachi, Japan). The urinary protein excretion was measured using biuret method.

2.4. Histopathological examination

The kidney tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections (3 μm) were stained with hematoxylin-eosin (HE) and Masson's trichrome to evaluate histopathological injury. The glomerulosclerosis index was

Table 1
Primer sequence used in this study.

Targrt gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
TGF-β1	TATAGCAACAATTCCTGGCGTTAC	TGTATTCCGTCTCCT TGG TCA
Smad2	AGTGTTTGCCGAGTGCCTAAGTG	CCTCAAAACCCTGGTTAACAGACTG
Smad3	CATTACCATCCCCAGGTCAC	CGTAACTCATGGTGGCTGTG
Smad7	AGGCTCTACTGTGTCCAA	ACTCTTGTTGTCCGA ATTGA
β-actin	CACCCGCGAGTACAACCTTC	CCCATACCCACCATCACACC

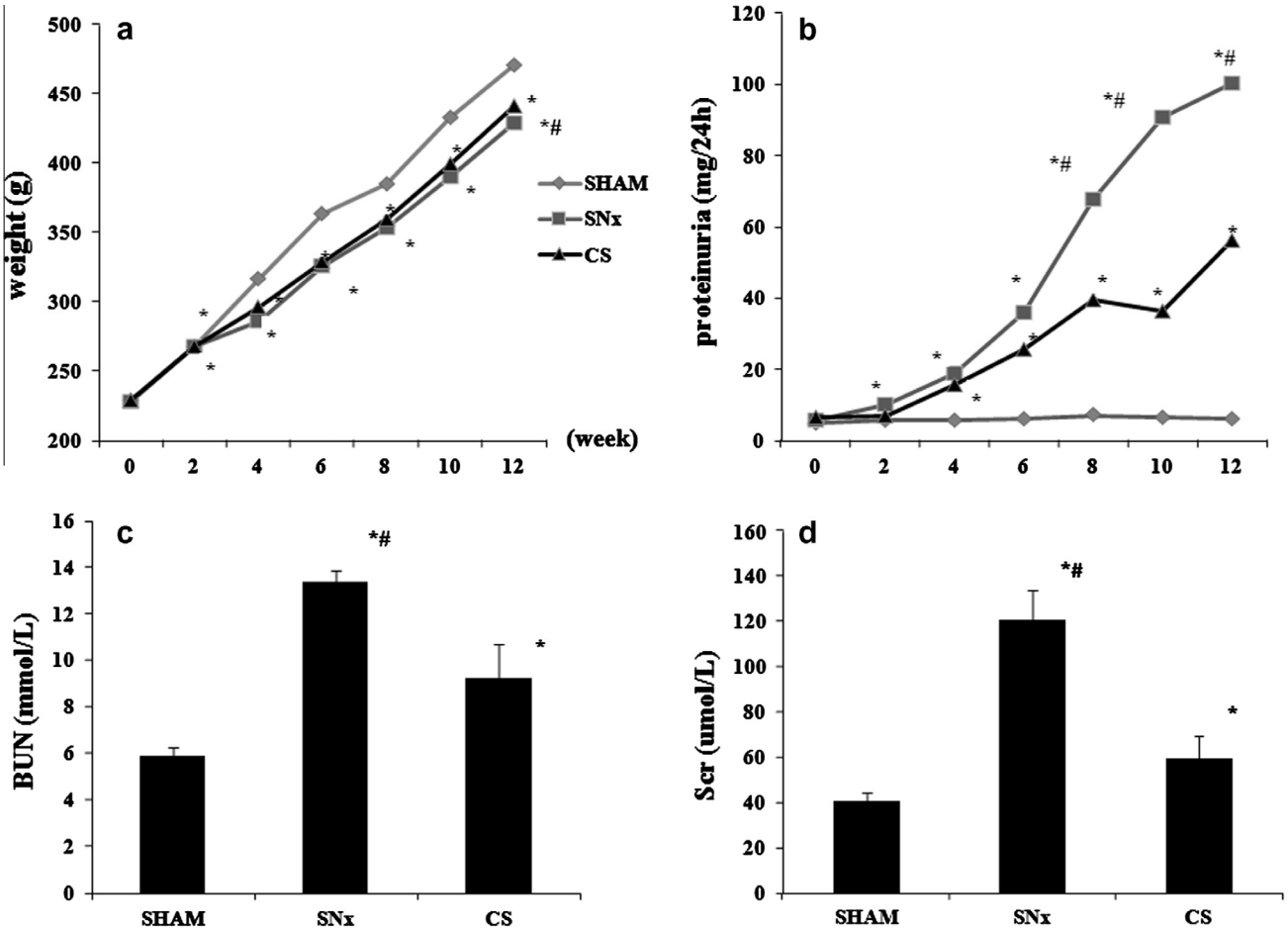

Fig. 1. The influence of *Cordyceps sinensis* on change of body weight, urinary protein and renal function in SNx rats. Time course of body weight (a) and urinary protein (b). Changes of BUN (c) and Scr (d). Data are presented as mean ± SD ($n = 8$ in each group). $^{*}p < 0.05$ versus SHAM group, $^{\#}p < 0.05$ versus CS group.