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Original Research Article

A study on the mechanism of cordycepin in regulating autophagy and alleviating renal tubular interstitial fibrosis

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Abstract

Purpose: *To elucidate the role of cordycepin in modulating autophagy and mitigating renal tubular interstitial fibrosis in a rat model of unilateral ureteral obstruction (UUO).*
Methods: *Forty male Sprague-Dawley (SD) rats were assigned to four groups: control, sham, UUO and cordyceps-treated groups (10 rats per group). The UUO and cordyceps groups underwent surgery to induce unilateral ureteral obstruction. The cordyceps group received intravenous cordycepin (10 mg/kg) daily for 14 days, while the control and UUO groups received normal saline. Histopathological examination, assessment of fibrosis markers (α-SMA, collagen III) and autophagy markers (Atg5, LC3II/I) were conducted.*
Results: *The UUO group exhibited significant tubular damage and interstitial fibrosis, with elevated serum levels of pro-inflammatory and oxidative markers ($p < 0.05$). Cordycepin treatment attenuated these pathological changes, evidenced by reduced fibrosis, inflammation and oxidative stress. Enhanced autophagic activity was observed in the cordycepin group, suggesting a potential mechanism of its renoprotective effect.*
Conclusion: *Cordyceps is effective in inhibiting renal tubulointerstitial fibrosis, potentially through the activation of autophagy and reduction of inflammation and oxidative stress. Future studies should focus on unraveling the specific molecular mechanisms of cordycepin's action and assessing its applicability in CKD treatment.*

Keywords: *Cordycepin, Autophagy, Oxidative stress injury, Inflammatory damage, Tubulointerstitial fibrosis*

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INTRODUCTION

Chronic kidney disease (CKD), a significant global health concern, is marked by progressive renal structural deterioration and functional impairment [1]. Central to its pathology are glomerulosclerosis, renal interstitial fibrosis, tubular atrophy and a reduction in capillary density, with tubulointerstitial fibrosis (TIF) identified as a pivotal aspect of the disease process [2]. Notably, TIF is not just a pathological marker but a critical determinant of the long-term prognosis in CKD patients [3,4]. Interventions that effectively mitigate TIF have the potential to significantly improve patient outcomes [5].

Recent study has shed light on the therapeutic benefits of traditional Chinese medicine in CKD management [6]. Specifically, Chongcaoyishen Decoction has demonstrated promising results in enhancing renal function and decelerating the progression of TIF [7]. Within this herbal concoction, cordycepin emerges as a primary active component, suspected to play a crucial role in delaying renal interstitial fibrosis, as substantiated by existing literature [8,9].

This current study delves into the mechanistic aspects of cordycepin's action in CKD [10]. It is hypothesized that cordycepin may exert its renoprotective effects by modulating autophagy pathways, thereby inhibiting collagen synthesis and attenuating the process of renal tubulointerstitial fibrosis. This simultaneously reduces inflammatory and oxidative stress responses within renal tissues [11]. This investigation therefore aims to unravel the therapeutic potential of cordycepin in CKD, particularly in mitigating TIF, thus contributing to the expanding body of knowledge in nephrology and potentially informing future clinical applications.

EXPERIMENTAL

Animals

This study utilized 40 male Sprague–Dawley rats (weighing between 180 and 220 g, specific pathogen-free grade) sourced from the Laboratory Animal Center of Zhejiang Chinese Medical University (SYXK 2021-0012, Hangzhou, China). The rats were housed under controlled conditions, with a 12 h light/dark cycle, relative humidity between 50 and 70 %, and a constant room temperature (22 ± 2 ℃). The animals had free access to a standard rat diet (comprising 18 % protein, 58 % carbohydrate and 4.5 % fat) and water. The Ethics Committee of Animal Experiments approved the experimental protocol at Zhejiang Chinese Medical University (approval no. IACUC-20220913-26, Hangzhou, China). The procedures conformed to the established guidelines for laboratory animal care [12].

Pharmaceutical and surgical interventions

Following acclimatization, rats were randomly allocated into four groups viz: control, sham-operated, unilateral ureteral obstruction (UUO) and cordyceps-treated groups. The UUO and cordyceps groups underwent a surgical procedure to induce UUO, establishing a CKD model as per established protocols [13]. Post-surgery, the cordyceps group received cordycepin intravenously (Yuanye, CAS No.73-03-0, Shanghai, China) at 10 mg/kg/day for 14 days, while the sham and UUO groups received equivalent volumes of saline. On day 14, anesthesia was administered using 2.0 % sodium pentobarbital (30 mg/kg), and blood and kidney samples were collected. These samples underwent extensive analysis, including histopathology, enzyme-linked immunosorbent assay (ELISA), immunohistochemistry, transmission electron microscopy and western blot, to evaluate the therapeutic impact of cordycepin in CKD.

Histological analysis

Renal tissues from rats were processed for histological analysis. Following fixation in 10 % formalin, dehydration and paraffin embedding, 3 μm tissue sections were stained with hematoxylin and eosin (H&E) for general morphological assessment and Masson's trichrome for collagen detection. Microscopic evaluation was performed on three randomly selected fields in each section, ensuring unbiased sampling. Histological evaluations utilized a Digital pathological section scanning system (Leica) and a Digital imaging system (GE). ImageJ software was used for collagen quantification in Masson's trichrome-stained sections to determine the extent of tubulointerstitial fibrosis.

Immunohistochemistry

De-paraffinized and rehydrated renal sections were subjected to immunohistochemistry. After blocking endogenous peroxidase with 3 % hydrogen peroxide, sections were incubated with primary antibodies (Thermo Fisher) against α-smooth muscle actin (α-SMA; 1: 100) and collagen type III (Col-III; 1: 100). Following overnight incubation and subsequent secondary antibody application, antigen retrieval was conducted using Tris-EDTA butter. Staining development was achieved using DAB and hematoxylin, and images captured with a transmission electron microscope (JEOL). ImageJ software was employed to quantitatively analyze protein expression levels [14].

Western blotting analysis

Kidney tissues were processed for Western blot analysis. Homogenization was performed in radioimmunoprecipitation assay (RIPA) lysis buffer augmented with Benzonase nucleases and proteases as well as phosphatase inhibitor. Post-homogenization, the supernatants were subsequently extracted to determine protein concentrations. Proteins were resolved using

SDS-PAGE in a 10 % gel and transferred onto a PVDF membranes. Membranes were incubated with primary antibodies against GAPDH (1: 1000), α-SMA (1: 100), COL-III (1: 100) and LC3 (1: 1000), followed by species-specific secondary antibodies. Detection was achieved through enhanced chemiluminescence and ImageLab software was utilized to quantify protein expression.

Statistical analysis

Data analysis was performed using IBM-SPSS statistics software version 26.0. All continuous types of measurement data are presented as mean ± standard deviation (SD). One-way ANOVA was used to compare the differences between groups and Tukey's analysis was used to compare the two groups. Variations in the full text were deemed to be statistically significant at $p < 0.05$.

RESULTS

Cordycepin alleviated renal tissue injury

This study focused on examining the renoprotective effects of cordycepin in chronic kidney disease (CKD) rat model induced by unilateral ureteral obstruction (UUO). One of the significant indicators of CKD in the UUO model is a notable renal enlargement, often apparent by the 14th day post-obstruction. Histopathological analyses were performed on renal sections to evaluate the morphological alterations and tissue damage in the kidneys. These sections, stained with H&E and Masson staining, revealed critical pathological characteristics, including increased renal interstitium size, inflammatory cellular infiltration, extracellular matrix hyperplasia and significant interstitial fibrosis, as illustrated in Figure 1. Interestingly, cordycepin administration effectively mitigated these pathological manifestations, underscoring its potential to reduce renal injury associated with UUO-induced CKD.

Cordycepin alleviated renal tubular interstitial fibrosis

In a unilateral ureteral obstruction (UUO) rat model, the study evaluated cordycepin's influence on TIF. Immunohistochemistry (IHC) analyses demonstrated increased COL-III and α-SMA levels in UUO rat kidneys compared to controls (Figure 2). Cordycepin administration significantly reduced these fibrosis markers, underlining its potential to attenuate TIF.

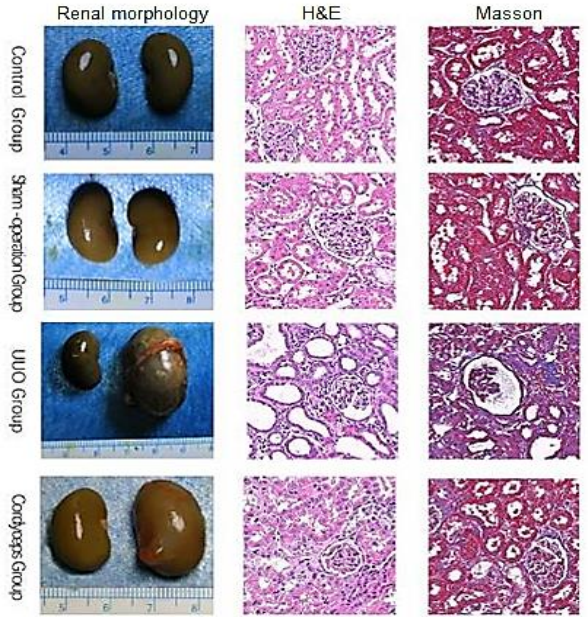

Figure 1: Renal morphology and histopathological changes of renal tissue from UUO rats, as evidenced by H&E and Masson staining (200x)

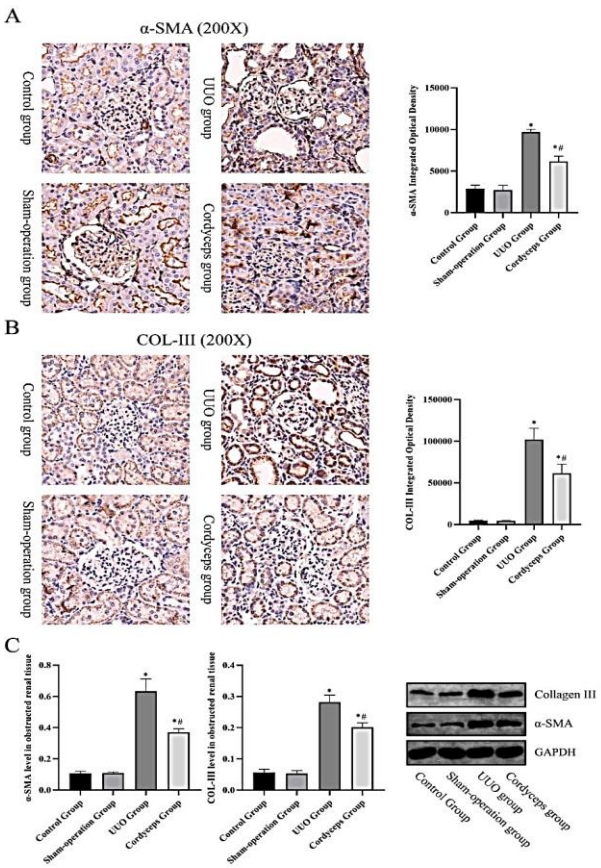

Figure 2: Assessment of renal tubulointerstitial fibrosis level. (A) IHC staining for α-SMA in renal tissue; (B) IHC staining for COL-III in renal tissue; (C) WB analysis of α-SMA and COL-III in obstructive renal tissues. *$P < 0.05$ versus sham-operation group

Cordycepin alleviated inflammatory damage and oxidative stress injury

The effect of cordycepin on inflammation and oxidative stress in rats with unilateral ureteral obstruction (UUO) are depicted in Figure 3. In UUO rats, there was a significant increase ($p < 0.05$) in pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and a concurrent decrease in anti-inflammatory cytokines (IL-10 and IL-12) compared to sham-operated controls. Treatment with cordycepin effectively reversed these inflammatory trends, reducing the levels of pro-inflammatory cytokines and enhancing those of anti-inflammatory cytokines. Furthermore, cordycepin exhibited a protective effect against oxidative stress in UUO rats. This was evidenced by increased serum malondialdehyde (MDA) levels and reduced antioxidant enzymes (SOD and GSH) in untreated UUO rats. Post-treatment, cordycepin significantly reduced MDA levels while augmenting SOD and GSH concentrations, showcasing its potential to alleviate oxidative stress.

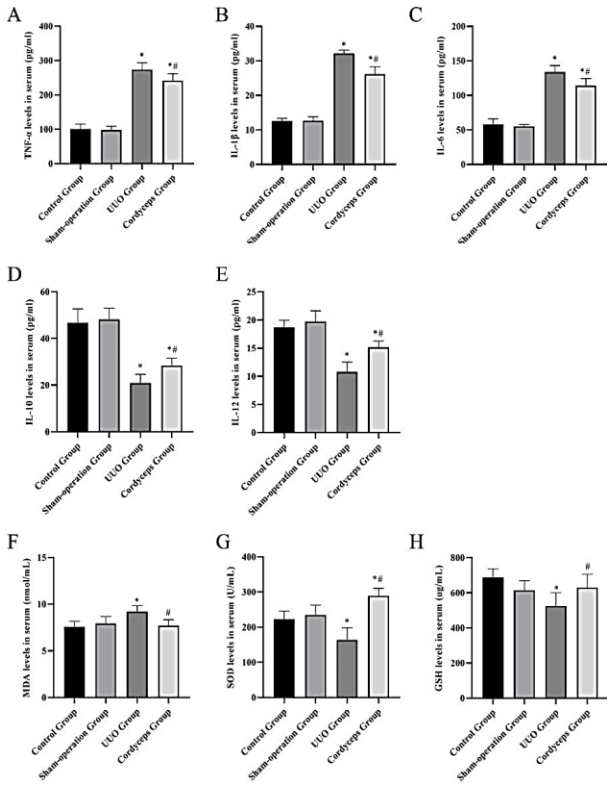

Figure 3: Comparative analysis of serum inflammatory and oxidative stress biomarkers in UUO rats. Tumor necrosis factor (TNF-α) (A), Interleukin-1 beta (IL-1β) (B), Interleukin-6 (IL-6) (C), Interleukin-10 (IL-10) (D), Interleukin-12 (IL-12) (E), and oxidative stress markers Malondialdehyde (MDA) (F), Superoxide Dismutase (SOD) (G) and Glutathione (GSH) (H). *$P < 0.05$ versus sham-operation group

Cordycepin further activates the autophagy in renal tissue

The impact of cordycepin on autophagic processes in renal tissues of rats with unilateral ureteral obstruction (UUO) are presented in Figure 4. Transmission electron microscopy (TEM) analyses identified a baseline presence of autophagosomes in normal renal tubular epithelial cells. In UUO rats, an enhanced number of autophagosomes was observed, further amplified by cordycepin treatment. Additionally, the expression of autophagy-related proteins was examined. Relative to the sham-operated group, UUO rat renal tissues exhibited a significant increase in autophagy-related protein 5 (Atg5) expression and a higher microtubule-associated protein 1A/1B-light chain 3 (LC3II/I) ratio. These changes were notably more distinct in the cordycepin-treated rats, corroborating the TEM findings.

DISCUSSION

Tubulointerstitial fibrosis (TIF) is a pivotal pathological aspect of chronic kidney disease (CKD) progression, critically influencing patient outcomes [10]. Traditional treatment approaches often inadequately address TIF, potentially leading to end-stage renal disease (ESRD) and necessitating interventions like dialysis or transplantation [15-18]. Recent advancements in traditional Chinese medicine (TCM), specifically Chongcaoyishen Decoction, have demonstrated its efficacy in decelerating TIF, with cordycepin emerging as a significant active component [5]. This study corroborates these insights, showcasing cordycepin's ability to attenuate renal pathology in UUO rats. In this study, reduced fibrosis markers and collagen fiber accumulation were observed in renal tissues, supporting the idea that cordycepin can hinder CKD-related pathological progression.

The development of renal TIF in CKD is primarily driven by inflammation and oxidative stress [19]. The results identified marked changes in these parameters in the UUO model, including elevated levels of TNF-α, IL-1, IL-6 and MDA, along with reduced anti-inflammatory cytokines (IL-10, IL-12) and antioxidant enzymes (SOD and GSH). Post-treatment with cordycepin, these indicators demonstrated significant improvement, further supporting its role in modulating inflammatory and oxidative mechanisms to alleviate TIF in CKD [29]. In the context of tubulointerstitial fibrosis (TIF) in chronic kidney disease (CKD), reactive oxygen species (ROS) generated from damaged mitochondria is specifically targeted and degraded by autophagy, a cellular process

that can be activated in obstructive kidney tissue [20]. However, this activation of autophagy is generally a passive cellular response and offers limited protective effects [21]. Previous study confirmed that Chongcaoyishen Decoction, which contains cordycepin as its main active ingredient, activated autophagy in the epithelium and reduced mitochondrial injury, indicating that this decoction enhanced the protective autophagy response during TIF [5].

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses have identified multiple functional pathways and cellular functionalities closely associated with autophagy in the pharmacological network of Chongcaoyishen Decoction, some of which are directly involved in autophagy regulation [22,23]. This indicates a significant role of Chongcaoyishen Decoction, and by extension cordycepin, in autophagy modulation. This study utilized transmission electron microscopy to observe autophagic structures in the renal tissues. A significant increase in autophagosomes and autolysosomes in the unilateral ureteral obstruction (UUO) group was observed, which was further amplified in the cordycepin-treated group. This observation was supported by changes in autophagy-related protein 5 (Atg5) expression and the LC3II/I ratio, aligning with the electron microscopy findings. These results confirms that autophagy in renal tubular epithelial cells undergoing tubulointerstitial fibrosis was considerably elevated compared to normal conditions.

By enhancing autophagy, cordycepin amplifies this passive defense mechanism, promoting organelle degradation and ROS decomposition, thereby reducing oxidative stress and inflammatory damage and delaying TIF progression. Still, the current study does not address the specific molecular pathway through which cordycepin regulates autophagy, nor does it exclude the possibility of cordycepin exerting its effects on TIF through non-autophagy-related mechanisms. Further study is warranted to determine the detailed mechanisms of cordycepin in modulating TIF and the role of autophagy in renal interstitial fibrosis.

CONCLUSION

The findings of this study substantiate the efficacy of cordyceps, especially its active ingredient cordycepin, in mitigating renal tubulointerstitial fibrosis in a unilateral ureteral obstruction (UUO) rat model. The results also suggest that cordycepin's renoprotective effects are largely due to its activation of autophagy pathways, which are instrumental in reducing inflammation and oxidative stress in renal tissues. These findings underscore cordycepin's potential as a therapeutic agent in chronic kidney disease (CKD), particularly for managing and potentially delaying the progression of tubulointerstitial fibrosis. Future studies should focus on unraveling the specific molecular mechanisms of cordycepin's action and assessing its applicability in CKD treatment.

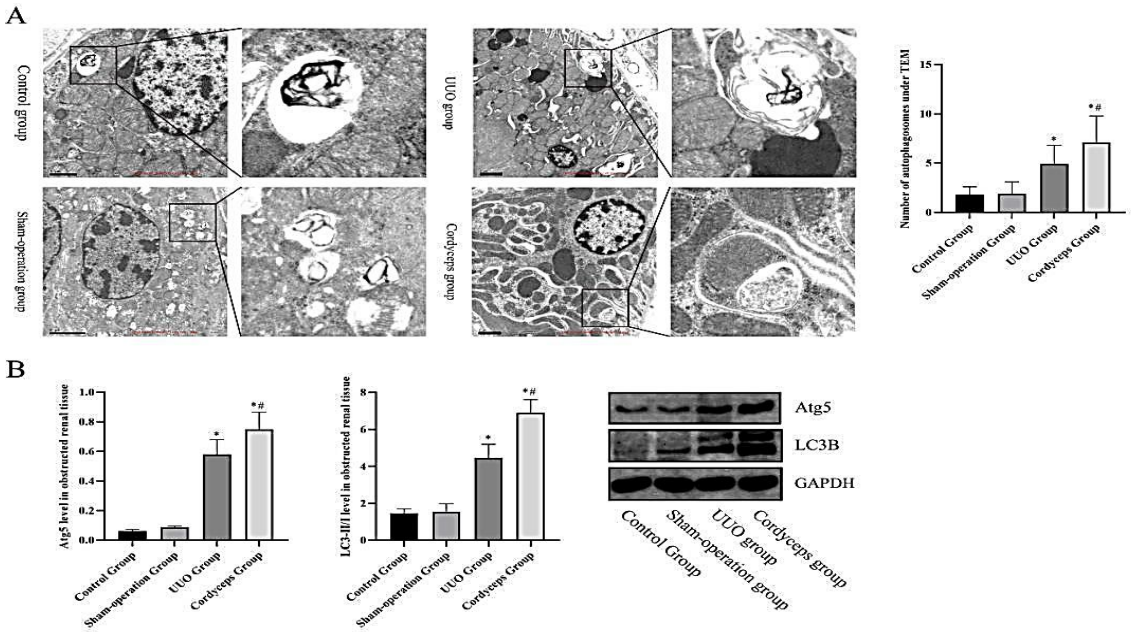

Figure 4: (A) Electron microscopy-based quantification of autophagosomes in renal tubular epithelial cells; (B) Measurement of autophagy-related protein 5 (Atg5) expression and LC3II/I ratio in renal tissue. *P < 0.05 versus sham-operation group

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ke Sun and Junfeng Fan conceived and designed the study, and drafted the manuscript. Ke Sun, Zhenliang Fan and Junfeng Fan collected, analyzed and interpreted the experimental data. Zhenliang Fan and Junfeng Fan revised the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors approved the final manuscript for publication.

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