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Research Paper

Mycelia glycoproteins from *Cordyceps sobolifera* ameliorate cyclosporine-induced renal tubule dysfunction in rats

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ABSTRACT

Ethnopharmacological relevance: *Cordyceps sorbolifera* has been used in Traditional Chinese Medicine for improving the renal function. Cyclosporine A (CsA) is an important immunosuppressive agent in the prevention of renal allograft rejection, but long-term usage of CsA could lead to chronic nephrotoxicity and renal graft failure. The study was aimed to investigate whether the mycelia glycoproteins of *Cordyceps sobolifera* (CSP) exert prevention effects on CsA-induced nephrotoxicity.

Materials and methods: Sprague-Dawley (SD) rats were randomly assigned into four groups ($n=6$ per group): normal saline (control group), CSP group, CsA group, and CSP-CsA group (CsA combined treatment with CSP). Glomerular and tubular functions were assessed and histological studies were performed.

Results: CSP, prepared by hot water extraction, ethanol precipitation and membrane dialysis, was found to be composed of three glycoproteins with average molecular weights of 543, 31, and 6.3 kDa, respectively. CsA impaired urea clearance and creatinine clearance were significantly improved by concomitant administration of CSP. TUNEL histochemical stain revealed that CSP significantly decreased CsA-induced apoptosis in renal tubular cells. The reducing effect of caspase-3 activation by CSP was suggested through the over-expression of the anti-apoptosis protein Bcl-2 in renal tubule cells. In assessment of CSP protection of renal tubule function, we found that CSP restored CsA induced magnesium wasting by increasing the magnesium reabsorption channels TRMP6 and TRMP7.

Conclusion: The results suggested that CSP had a significant suppressive activity on CsA-induced apoptosis and protective activity against nephron loss possibly *via* its restoring activity by increasing the magnesium reabsorption channels TRMP6 and TRMP7 on CsA induced magnesium wasting.

1. Introduction

Cyclosporine A (CsA) is a calcineurin inhibitor used in the prevention of allograft rejection and in clinical practice for the treatment of autoimmune diseases. However, CsA toxicity is one of the major causes of chronic allograft nephropathy (CAN), which could lead to allograft failure (Naesens et al., 2009). CsA can induce cellular damage including fibrosis through the activation of the intrinsic apoptotic pathway and vasculopathy by constriction of efferent and afferent arterioles of the glomerulus, leading to alterations in hemodynamic change and decreased glomerular filtration in the kidney (Bobadilla and Gamba, 2007; Shihab et al., 1999). Many strategies have been tested to improve hemodynamic impairment caused by CsA, such as blocking of the renin–angiotensin–aldosterone system (RAAS) and the calcium channel (Inigo et al., 2001). Other methods include the use of aldosterone or endothelin antagonists, thromboxane receptor antagonist, magnesium supplement or paricalcitol (Burdman and Bennet, 2008). However, none of these strategies have been proved effective in clinical applications. In order to achieve a higher allograft survival rate, a better understanding of CsA nephrotoxicity mechanisms and the development of different approaches are needed to reduce CsA nephrotoxicity in post-kidney transplant patients.

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Cordyceps is a genus that includes over 400 species, of which 3 species in the group *Cordyceps sinensis* have been used as traditional medicine to improve the renal function in China since the 17th century (Paterson, 2008). All three species (*Cordyceps sinensis*, *Cordyceps sobolifera* and *Cordyceps cicadae*) have a common characteristic in renal protection effects (Paterson, 2008). *Cordyceps sinensis* can ameliorate CsA nephrotoxicity in patients with kidney transplants (Xu et al., 1995). *Cordyceps cicadae* showed renal protection effects in chronic kidney disease (CKD) patients receiving Western medicine (Wojcikowski et al., 2006). in vitro study revealed that *Cordyceps sobolifera* (CS) could protect mesangial cells from sclerosis (Wang and Chen, 2006). In in-vivo animal studies, CS inhibited renal fibrosis and prevented nephrosclerosis in a 5/6 nephrectomy model (Jin et al., 2006; Zhu et al., 2011). More recently, the water extract of CS provided protective effects against lipopolysaccharide-induced nephrotoxicity in rats (Wu et al., 2011). However, despite many studies have been conducted on CS functions, their protective effects from CsA nephrotoxicity remain to be explored. Due to the polysaccharides were the major constituents of CS, we hypothesized that *Cordyceps sobolifera* polysaccharides (CSP) may have protective effects against acute CsA nephrotoxicity. Therefore, the current study was performed to investigate whether the biochemical changes and the renal dysfunctions induced by administration of CsA in rats could be improved by concomitant administration of CSP.

2. Materials and methods

2.1. Preparation of Cordyceps sobolifera (CS) mycelia

The freeze-dried mycelia were prepared as previously described (Wu et al., 2011) and provided by the Biotechnology Center of Grape King Inc., Chung-Li City, Taiwan. The voucher specimens of the materials were deposited at the laboratory of Biological Products of the Institute of Biotechnology, Hungkuang University, Taiwan. In brief, Cordyceps sobolifera (BCRC 37801) obtained from Food Industry Research and Development Institute (Hsinchu, Taiwan), was grown on potato dextrose agar and was incubated at 25 °C for 4 days. Thereafter, a piece of 5 mm × 5 mm *Cordyceps sobolifera* agar culture was inoculated into a 2 l Hinton flask with 1 l medium consisting of sucrose 2.5% sucrose, 1.2% soybean powder, and 0.8% yeast extract; pH was adjusted to 5.0 with 1 M of citric acid. The medium was cultivated at 25 °C in a 100 rev/min rotary shaker for 3 days and then scaled up to a 500 l fermenter with agitation for another 3 days as seed culture. The seed culture was then inoculated into a 10-ton fermenter and cultured for 4 days under the same conditions. The light mocha whole broth was then freeze-dried under vacuum, ground to powder and stored at room temperature.

2.2. Preparation of water soluble polysaccharides

Water soluble polysaccharides from mycelia of CS were prepared as previously reported (Chen et al., 2007) and as the following procedures. The defatted mycelia powder (1 kg) obtained by supercritical carbon dioxide extraction under the pressure of 5000 psi at 60 °C for 2 h was extracted with reflux three times with 20 l of double-distilled water (DDW) at 90 °C with constant stirring at 400 rpm for 2 h. The extracts were filtered with aspiration after cooling. To the filtrate was concentrated *in vacuuo* at 4 °C by rotary evaporation and then a 2-fold volume of ethanol (95%) was added to precipitate the water-soluble polysaccharides, which were collected and further purified in distilled water. Finally, the water-soluble polysaccharides were precipitated on addition of a 3-fold volume of ethanol (95%) and then collected and lyophilized (CSP). The phenol-sulfuric acid method (Masuko et al., 2005) and the Lowry method (Hartree, 1972) were used to determine the contents of polysaccharide and protein, respectively. The contents of polysaccharide and protein in CSP were found to be 0.99 ± 0.12 and 96.21 ± 1.25% of dry matter, respectively. Obviously, carbohydrate groups in the isolated products were covalently attached to proteins to form glycoproteins not only existed as the pure carbohydrate structure.

2.3. Analysis of CS glycoproteins

High performance size-exclusion chromatography (HP SEC) was used to analyze the obtained CSP by using the tandem columns of PolySep-GFC-P (75 × 7.8 mm) and PolySep-GPC-P 4000 (300 × 7.8 mm, Phenomenex, Torrance, CA. USA) serially coupled to ultraviolet (UV, 280 nm) and evaporative light scattering detectors (ELSD, SEDERE, SA, Alfortville, Cedex, France). The ELSD system was operated with the following settings: a drift tube temperature of 50 °C, a gain of 5, and a nebulizer nitrogen gas pressure of 2.3 bar. Deionized water was used as the mobile phase with the flow rate at 0.8 ml/min. The column oven temperature was 40 °C. The sample injection volume was 20 μl. Molar mass was determined using an ELSD analysis by standard calibration with 8 pullulan standards (Showa Denko K. K., Shodex, Japan) including the molecular mass of 788, 404, 212, 112, 47.3, 22.8, 11.8 and 5.9 kDa, respectively).

2.4. Animals study

Seven-to-eight-week-old Sprague-Dawley (SD) rats, weighing between 200 g and 250 g, were used in this study. They were housed in a room with controlled temperature (22 ± 2 °C), humidity (55%) and light (12 h light–dark cycles). Animals were fed standard laboratory chow and given reverse osmosis water ad libitum. The study protocol and procedures were approved by the Animal Experimental Committee of Taichung Veterans General Hospital. Four groups of eight rats each were randomly assigned to the CSP group, CsA group, CSP-CsA group and control group, respectively, for nasogastric tube feeding. The CSP group received CSP (500 mg/kg) for 7 days; the CsA group received CsA (40 mg/kg, Novartis Pharma, Basel, Switzerland) for 7 days. In the CSP-CsA group, animals were treated with CsA first and 6 h later, the CSP was administrated in the same day for 7 days. Animals in the control group received 0.9% normal saline. Each treatment was given once daily at a volume of 10 ml/kg. Before sacrifice, the animals were allocated into metabolic cages for 24 h for urine collection. The amount of urine was measured and stored at −20 °C until analysis. The animals were sacrificed under ether anesthesia. Blood samples were taken by cardiac puncture and collected in non-heparinized tubes. Plasma was separated by centrifugation for 10 min at 5000 rpm (4 °C) and stored at −20 °C until analysis. After blood sampling, both kidneys were quickly isolated and washed with ice-cold isotonic saline solution. Half of the kidney was dissected and fixed in 4% buffered paraformaldehyde at room temperature, dehydrated with alcohol and embedded in paraffin. The other half of the kidney was dissected and frozen at −80 °C until analysis.

2.5. Biochemical analysis

All plasma and urine biochemical parameters including creatinine, blood urea nitrogen, calcium, phosphate, magnesium and sodium were analyzed by a Sequential Multiple Auto analyzer (Beckman, Synchron Clinical Systems, California, USA) at the ISO-approved biochemistry department of Taichung Veterans General Hospital. The determination of the following parameters: the fractional excretion of Mg^{+2} (FE Mg), Na^{+} (FE Na), Ca^{+2} (FE Ca) and uric acid (FE uric acid) was also included in this study.