Journal of Ethnopharmacology 186 (2016) 125–135

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Astragalus polysaccharides decrease muscle wasting through Akt/mTOR, ubiquitin proteasome and autophagy signalling in 5/6 nephrectomised rats

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ARTICLE INFO

Article history:
Received 16 October 2015
Received in revised form
17 February 2016
Accepted 31 March 2016
Available online 2 April 2016

Keywords:
Muscle wasting
Astragalus polysaccharides
Chronic kidney disease
Protein metabolism

ABSTRACT

Ethnopharmacological relevance: Existing evidences suggest that *Radix Astragali* and its polysaccharides composition (APS) can improve muscle mass, but the mechanisms need more research.
Aim of the study: In this study, we aimed to examine the effects of APS on muscle wasting at molecular level in 5/6 nephrectomised rats.
Materials and methods: We performed 5/6 nephrectomy or sham operation in 160 6-week-old Sprague–Dawley rats, and feed animals with or without 2% APS for 155 days. After treatment, we compared the change of weight, muscle fibre, protein metabolism, pro-inflammatory factors (TNF-α, IL-15, CRP) and oxidative factors (MDA, SOD) among each group. In addition, we detected the Akt/mTOR, ubiquitin proteasome, autophagy signalling and AA transporters in vivo and in vitro.
Results: Data in vivo show 2% APS could alleviate weight loss and improve protein metabolism in nephrectomised rats. The levels of serum pro-inflammatory factors and oxidative factors were restored by APS treatment. In molecular levels, APS restored Akt/mTOR, MAFbx, MuRF1, Atg7, LC3B-II/LC3B-I and SLC38A2 which changed in nephrectomised rats. Data in vitro show the optimal dose of APS is 0.2 mg/mL, and SLC38A2 siRNA attenuated the effects of 0.2 mg/mL APS on atrophy and autophagy.
Conclusions: Our results suggested APS could improve muscle wasting through Akt/mTOR, ubiquitin proteasome and autophagy signalling, and SLC38A2 may be one of potential targets.

1. Introduction

Muscle wasting always occurs in patients with chronic kidney disease (CKD), particularly those with end-stage renal disease (ERSD) (Wang and Mitch, 2014; Workeneh and Mitch, 2010). Mortality is independently related to loss of muscle mass in CKD patients (Rajan and Mitch, 2008). CKD-related muscle wasting causes malnutrition due to low protein intake and leads to protein energy wasting (PEW) (Buscher et al., 2010; Stenvinkel et al., 2004), which has numerous causes, including persistent inflammation, acidosis, multiple endocrine disorders and dialysis (Wang and Mitch, 2014). This, simply altering the diet cannot fully correct the excessive morbidity and mortality in CKD patients (Stenvinkel et al., 2004). Due to the complex etiology, there is still no optimal treatment for muscle wasting in CKD. The imbalance between the anabolic and catabolic rates is responsible for the occurrence and progression of PEW. In CKD progression, protein catabolism is induced by proteolytic pathways, such as liposomal, ubiquitin-proteasome dependent signalling (UPS) and autophagy (de Palma et al., 2008; Galluzzi et al., 2014; Sala et al., 2014). The low anabolic rate is commonly attributed to attenuated mTOR

Abbreviations: AA, amino acid; Akt, thymoma viral proto-oncogene; APS, *Astragalus* polysaccharides; Atg, autophagy associated gene; CKD, chronic kidney disease; CRP, C-reaction protein; ELISA, enzyme-linked immunosorbent assay; ERSD, end-stage renal disease; GC, gas chromatography; HPGFC, high performance gel filtration chromatography; IL, interleukin; LNAA, especial large neutral amino acid; MAFbx, muscle atrophy F-box; MDA, malondialdehyde; MDC, monodansylcadaverine; mTOR, mammalian target of rapamycin; MuRF1, muscle ring finger 1; PCR, polymerase chain reaction; PEW, protein energy wasting; siRNA, small interfering RNA; SOD, superoxide dismutase; TA, tibias anterior; TNF, tumour necrosis factor; UPS, ubiquitin-proteasome dependent signaling

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http://dx.doi.org/10.1016/j.jep.2016.03.068
0378-8741/

signalling (Gordon et al., 2013; Wang et al., 2009). These aspects of protein metabolic signalling may be regarded as potential therapeutic targets for CKD-related muscle wasting.

Radix Astragali is the root of *Astragalus membranaceus (Fisch.) Bunge*, which is a popular medicinal and food plant in China, where it is known as Huang-qi. According to records of Chinese materia medica, Huang-qi was used to tonify Qi, and it has been used as therapy for "Wei Zheng" , a term for skeletal muscle fatigue and wasting (Gao et al., 2005; Zhou and Mei, 2014). The effect of *Radix Astragali* on muscle fatigue has been proven by a series of studies (Kuo et al., 2009; Yeh et al., 2014; Zhang et al., 2015a), but its medical use for treating muscle wasting still lacks experimental evidence. A previous study showed that *Radix Astragali* improves the protein levels of muscle in nephrotic patients (Li et al., 1995). However, the active ingredients and mechanisms remain unclear. The major bioactive constituents of *Radix Astragali* are astragloside, triterpene glycosides, polysaccharides, flavonoid, saponin, amino acid, alkaloid, organic acid, microelement etc (Liu et al., 2015b; Movafeghi et al., 2010). Previous studies reported some benefit effects of polysaccharides (APS), saponins (ASS), and flavonoids (ASF) fractions on muscle disorders such as insulin resistance and fatigue (Zhang et al., 2015b). APS are the polysaccharide components of the water extracted from *Astragalus* roots. They are made from the components rhamnose, arabinose and glucose (Fu et al., 2013). Previous studies have reported that APSs have beneficial bioactivities on some disorders, including inflammation, oxidative stress and insulin resistance (Huang et al., 2013; Liu et al., 2015a, 2010, 2014; Mao et al., 2009), which are closely associated with the CKD-PEW process. Furthermore, our recent studies have shown that the polysaccharides in the water extract from *Astragalus* roots can reduce muscle atrophy in vitro by restoring the phosphorylation of mTOR and by suppressing myostatin (Liu et al., 2013; Lu et al., 2013a). Given this information, we speculate that APSs may be beneficial for patients with CKD-related muscle wasting.

In this study, we aimed to determine the effect of water-soluble polysaccharides (from *Astragalus mongholicus*) on muscle protein metabolism in a rat renal failure model. We also explored possible mechanisms for these effects. This research may help in the search for effective treatments for CKD-related muscle wasting.

2. Materials and methods

2.1. Animals

Five-week-old male Sprague–Dawley rats (obtained from the Experimental Animal Center of Southern Medical University, China, certification no. SCXK (Yue) 2011–0015) weighing 145–155 g were housed at a constant temperature with a 12/12-h light-dark cycle and less than 60 dB of noise. The rats were kept according to the guidelines for Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China, and all experimental procedures were approved by the Ethics Committee of Southern Medical University. Animals were allowed to adapt to their surroundings for 1 week with normal feeding based on semi-purified diet AIN 93-G before starting the experiments. Male rats were randomly assigned to either the 5/6 nephrectomised group or the sham-operated group. Each animal in the nephrectomised group underwent a 5/6 nephrectomy, consisting of ablation of two thirds of the mass of the left kidneys, followed by right unilateral nephrectomy after 1 week. In the sham-operated rats, a sham operation was performed according to previous methods (Wang et al., 2014a).

Table 1
Ingredient of experimental diets to rats (g/kg diets).

Components	Control	Model	M-APS	C-APS
Casein	220	220	220	220
Dextrin	379	379	379	379
Maize starch	140.5	140.5	120.5	120.5
APS	–	–	20	20
Sucrose	100	100	100	100
Soyabean oil	90	90	90	90
Mineral mix	35	35	35	35
Inherent fibre	20	20	20	20
Vitamin mix	10	10	10	10
L-Cystine	3	3	3	3
Choline hydrochloride	2.5	2.5	2.5	2.5
Gross energy, kcal/g	4.50	4.50	4.42	4.42

Note: APS, *Astragalus* polysaccharide.

2.2. APS preparation

Astragalus membranaceus (Fisch) Bunge was purchased from the Guangzhou Qingping Medicine Market (Guangzhou, China) and identified by the Department of Authentication of Chinese Medicine, Chinese Traditional Medicine (Shanghai, China) by comparison to the specimens deposited under the voucher number SHCTM-817.

APSs were extracted and purified with optimized techniques using direct water decoction according to previous methods (Mu et al., 2009). The APSs were purified using the Sevage method and column chromatography with a Sephadex G-200. The polysaccharide content was 95.25%, as determined by the phenol–sulphuric acid method. The weight average molecular mass of the APSs was measured by high-performance gel filtration chromatography (HPGFC); the results are shown in Supplementary Fig. 1. The individual monosaccharides from the polysaccharide fractions were identified by gas chromatography (GC); the results are shown in Supplementary Fig. 2 and Table 1.

2.3. Diet preparation and experimental design

Two dietary treatments were used in this study. The control diet was amended based on an AIN-93G diet. The control diet was fed to the sham-operated group (control group) and the 5/6 nephrectomised group (model group). The treatment diet consisted of the control diet with 2% APS substituted for maize starch, and this diet was fed to 40 of the 5/6 nephrectomised rats (M-APS group) and 40 of the sham-operated rats (C-APS group). To guarantee the comparability of each group, we ensured that all diets contained similar protein contents. The gross energy of each diet was greater than 4.4 kcal/g. The ratio of caloric density in the APS diets to other diets was kept to at least 98% by adjusting the carbohydrate-fat ratio of the AIN-93G diet. The ingredients and chemical composition of the diets are listed in Table 1. All rats were allowed to drink tap water ad libitum. All diets were prepared by the Guangdong Provincial Medical Laboratory Animal Center (Foshan, China). The duration of the study was 155 days. Food intake was determined daily. We define weight loss as an end event if weight gain did not reach 20% of the average weight gain of the control group for two consecutive weeks. (This standard was based on a receiver operating characteristic curve comparing weight loss and muscle pathology in a pre-experiment). Body weights, weight loss events and death were recorded weekly.

2.4. Myofibre cross-sectional area measurements and gradient screening of APSs

Myofibre cross-sectional area and C2C12 fibre size measurements were performed according previous experiments(Lu et al.,