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Ampelopsin attenuates the atrophy of skeletal muscle from D-gal-induced aging rats through activating AMPK/SIRT1/PGC-1α signaling cascade

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ABSTRACT

The atrophy of skeletal muscle is highly correlated with oxidative damage, excessive apoptosis and dysfunctional autophagy. Ampelopsin, a natural flavonoid, has multiple biological functions including anti-inflammatory, anti-oxidative, and hepatoprotective functions. Sprague-Dawley (SD) rats subjected to intraperitoneal injection of D-galactose (D-gal) at the dose of 150 mg/kg·d revealed an obvious atrophy of skeletal muscle with significantly reduced muscle mass/body mass ratio, cross-sectional area and fiber diameter of skeletal muscle in D-gal-induced aging rats when compared to normal control rats without D-gal administration for 6 consecutive weeks. In contrast, the combinatorial administration of D-gal at the identical dose and DHM at the dose of 100 or 200 mg/kg·d could alleviate the reduction of these hallmarks associated with the atrophy of skeletal muscle. In addition, D-gal administration could result in obvious apoptosis and impaired autophagy in skeletal muscle, which could be mitigated upon DHM treatment due to its role in decreasing ubiquitin and Atrogin-1/MAFbx and up-regulating AMPK and SIRT1 signal pathways. Therefore, DHM may be a potential candidate for the prevention and treatment of skeletal muscle atrophy associated aging process.

1. Introduction

Sarcopenia, a physiological loss in mass and functions of skeletal muscle during aging process, is a primary concern of increasing aged population all over the world. It is estimated that approximately 5–13% of elderly over 60 years old are affected by low mass and weak function of skeletal muscle [1]. The progressive mass loss of skeletal muscle can result in the impact on health, thereby leading to decreased physical activity and increased risk of falls or corresponding fractures in elderly [2]. In 2011, according to the estimation from World Health Organization (WHO), the population over 60 years old will present an expansion in the next decades, which will result in an increasing incidence of sarcopenia [3]. Although a series of intervention strategies, even including gene- and cell-based therapy, have been conducted, few drugs have been approved for the prevention or treatment of aging-related muscle atrophy. Therefore, the development of effective intervention strategy for aging-related or drug-induced muscle atrophy is highly desired.

The effects of intervention strategies such as nutritional supplements, caloric restriction and physical activity on the delay or attenuation of aging-related muscle wasting have been extensively explored, and regular physical activity or resistance exercise has been documented to slow down functional decline during aging process and even reverse muscle wasting. In humans, high-intensity resistance training can improve the strength and cross-sectional area of skeletal muscle [4]. Thus, regular exercise is an effective intervention strategy for the prevention and treatment of muscle wasting. However, a recent study has reported that only 2% of elderly participate in regular exercise. It may not be a feasible option for older people with physical impairments to prevent and treat muscle wasting by using exercise intervention. Therefore, novel and feasible interventions for preventing muscle wasting

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need to be considered, especially, those who cannot implement regular exercise. As muscle wasting is a multifactorial disease, multi-target interventions such as dietary supplements or pharmacological approaches may be the ideal candidates.

Ampelopsin, also called dihydromyricetin (DHM), is a natural flavonoid from Rattan tea as a medicinal and edible plant with a long application history in China [5]. Its chemical structure is shown in Fig. 1. DHM has been reported to possess anti-inflammatory, anti-oxidative, and anti-tumorigenic activities [6]. It has also been proven to alleviate the fatigue of skeletal muscle. Previous studies have demonstrated that DHM could improve physical performance under simulated high-altitude conditions [7]. Other studies have also reported its beneficial functions for the disorders of skeletal muscle such as insulin resistance and hyperglycemia-induced endothelial cell dysfunction through the induction of autophagy from the activated AMPK signaling pathway [8,9]. Irisin, a newly discovered myokine secreted into circulation system following proteolytic cleavage from fibronectin type III domain-containing protein 5 (FNDC5). It is activated in response to physical activity of skeletal muscle. In addition, the administration of DHM can stimulate the generation and secretion of irisin and improve serum irisin level in animal models and health people, suggesting that DHM can execute the function of mimic exercise to accelerate the generation and secretion of irisin [10]. These pioneer data provide DHM as a promising pharmacological treatment strategy of skeletal muscle-related disorders.

The mass maintenance of skeletal muscle mainly depends on the overall balance between protein synthesis and breakdown. So far, the mechanisms for the atrophy of skeletal muscle are still not clear. Protein degradation pathways, especially ubiquitin-proteasome system (UPS), play major roles in the atrophy of skeletal muscle, and have been documented as an obvious activation in skeletal muscle during aging process, which is believed to contribute to muscle wasting [11]. In skeletal muscle, ubiquitin E3 ligases such as Atrogin-1/MAFbx and MuRF1 are specifically expressed and significantly up-regulated in multiple models with the atrophy of skeletal muscle [12]. Thus, it is important and necessary for defining molecular mechanisms of skeletal muscle atrophy through regulating the expression of these proteins. Peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1α) is a master regulator of oxidative metabolism and the activation of PGC-1α has been demonstrated to prevent oxidative damage of skeletal muscle in young animals and exert anti-inflammatory function in aging skeletal muscle [13]. In addition, autophagy is an evolutionarily conserved process in eukaryotic organisms, defined as a catabolic pathway involving the degradation of cellular components via the lysosomal machinery [14]. Autophagy can promote cell survival by eliminating damaged organelles and protein aggregates. However, impaired or excessive autophagy can lead to the accumulation of damaged protein and intracellular organelles, and even promote cell death. A decline in autophagic function is a common trait of the aging process. Numerous studies have reported that the defects or dysfunction of autophagy are involved in the pathogenesis of sarcopenia [15–17], suggesting that pharmacological targeting to activate autophagy machinery may have therapeutic value. At present, a lot of anti-inflammatory drugs, hormones, and metabolic agents are being reported to have potential to prevent or slow down sarcopenia [18]. Furthermore, supplementation with antioxidants has been reported to be beneficial for the delay of aging process. In recent years, numerous traditional Chinese herbs have been also confirmed to possess potent anti-aging activity, and have gained considerable attentions as the potential candidates for the development of novel anti-aging strategies [19,20]. Therefore, DHM is expected to have an inhibitory effect on the atrophy of skeletal muscle, especially those who cannot implement regular or rigorous exercise. However, the inhibitory effect and underlying mechanisms of DHM on the atrophy of skeletal muscle have never been explored. D-Gal-induced oxidative stress rat is a good model for studying brain aging. Hence, we hypothesized that the atrophy of skeletal muscle from the oxidative stress of D-gal-induced aging process can be prevented by DHM. In the present study, we assessed the inhibitory effect of DHM on the atrophy of skeletal muscle in D-gal-induced aging rats, explored the impact of UPS signal pathway on the atrophy of skeletal muscle associated with D-gal-induced aging process and confirmed the involvement of AMPK/SIRT1/PGC-1α signaling pathway in potential prevention or control of atrophy of skeletal muscle during aging process through DHM intervention.

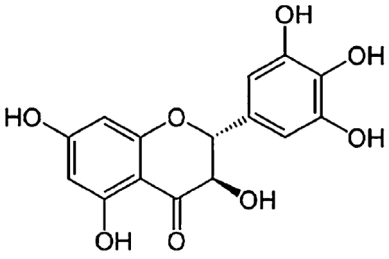

Fig. 1. Chemical structure of DHM.

2. Materials and methods

2.1. Drugs and reagents

Ampelopsin (CAS No. 27200-12-0) purified by high performance liquid chromatography (HPLC) (purity ≥ 98%) was purchased from Zelang Medical Technological Co. Ltd (Nanjing, China). D-gal was ordered from Sigma-Aldrich Corporation (St. Louis, USA). MDA and SOD kits were purchased from Jiancheng Company (Nanjing, China). Primary antibodies including Bcl-2, Bax, LC-3, Beclin1, p62, phosphor-AMPK, AMPK, SIRT1, phosphor-Akt (Ser473) and Akt, β-actin and GAPDH were purchased from Cell Signaling Technology (Danvers, MA, USA). Primary antibodies such as Fbx32 and MuRF1 were obtained from Abcam (Cambridge, MA, USA). Ubiquitin antibody was obtained from Bioss Inc. (Bioss, Beijing, China). All secondary antibodies for Western blot were purchased from Cell Signaling Technology (Danvers, MA, USA).

2.2. Animals and treatments

Totally 40 male Sprague-Dawley (SD) rats (age: 8 weeks; body weight: 160 ± 20 g; certificate No.: SCXK(e)2015-0018) were obtained from the Experimental Animal Center of Hubei Provincial Disease Control Center (Wuhan, China). The protocols were reviewed and approved by Institutional Animal Care and Use Committee at Wuhan Sports University. The rats were randomly divided into four groups including normal control group, D-gal model group, D-gal combined with DHM at the dose of 100 mg/kg·d and D-gal combined with DHM at the dose of 200 mg/kg·d groups with 10 rats in each group.

All rats were housed at a controlled room temperature (22 ± 2 °C) with a dark-light cycle (12 h: 12 h), and provided the accessibility to food and water *ad libitum*. After adapting to the new environment for 1 week, the rats from DHM groups were administered with DHM dissolved in distilled water at the designated dosages by gavage once a day at 8:00am for 6 consecutive weeks. The rats from the normal control group were administrated with distilled water without DHM. Except the normal control group, the rats from other groups were subjected to