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Platycodon grandiflorum-derived saponin attenuates the eccentric exercise-induced muscle damage

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ABSTRACT

Platycodon grandiflorum contains triterpenoid saponins, such as platycodin D and platyconic acid A, and acts as a multifold nutritious compound. Our previous research demonstrated that *Platycodon grandiflorum*-derived saponin (PS) improves high-fat diet-induced non-alcoholic steatohepatitis and inhibits osteoclast differentiation. The pivotal effects of PS on inflammatory mechanism were suppressed NF-κB and matrix metalloproteinase (MMPs). However, the effects of PS on skeletal muscle damage remain unknown. Therefore, we investigated whether PS protects against eccentric exercise-induced muscle damage. A significant reduction in eccentric exercise-induced muscle damage area and muscle damage related to the level of NF-κB p65 by PS was associated with the downregulation of ERK/p38/SMAD signaling. Eccentric exercise caused muscle damage by increasing the serum lactate dehydrogenase (LDH), creatinine kinase (CK) and C-related protein level. The serum LDH, CK and C-related protein level was significantly lower in the PS supplementation group compared with the control group. Moreover, PS was inhibited MMP-1, MMP-2 and MMP-9. PS protects against eccentric exercise-induced muscle damage. Together, these results provide a novel perspective on the biological function of PS against muscle damage.

1. Introduction

Skeletal muscles repeat the daily actions of walking, sitting, lifting, and bending. The mobilization of repetitive skeletal muscles in both daily life and intense physical activity increases the risk of micro- and macro-muscle damage. Eccentric treadmill exercise is known to induce muscle damage (Clarkson and Hubal, 2002). Muscle damage induced changes in morphology of skeletal muscle as structure protein disruption with inflammation (Nosaka et al., 2006). After muscle damage, tissue matrice metalloproteases (MMPs) degrade the extracellular matrix, deposit new extracellular matrix component, and regenerate tissue. Several MMP family have been reported to be involved in the progression of inflammation that occurs after injury or disease (Nissinen and Kahari, 2014). Inflammation is an essential step in the initiation and progression of tissue reconstruction, accompanied the degradation and remodeling of the extra cellular matrix scaffold to which MMPs are important contributors (Sbardella et al., 2012). When the magnitude of the inflammatory response exceeds a general level in response to traumatic damage, the extracellular matrix remodeling process is destroyed and can have deleterious effects on skeletal muscle. One example is an excessive increase in the activity of denatured MMPs, which impairs tissue destruction, cell invasion and tissue regeneration (Urso et al., 2012; Kieseier et al., 2001).

Natural sources contain phytochemical compound constituents of fruits, vegetables, and plants, which may attenuate inflammation-induced chronic diseases. *Platycodon grandiflorum* including steroidal saponins, flavonoids, phenolic acids, polyacetlyenes, and sterols (Zhang et al., 2015). The roots of *Platycodon grandiflorum* (Korean name, 'Doraji', Japanese name, 'Kikyo', and Chinese name, 'Jiegeng') has been used as a food and in traditional oriental medicine to treat adult disease such as asthma, bronchitis and pulmonary tuberculosis even as a sedative. In addition, *Platycodon grandiflorum* was used as an active ingredient and our previous studies reported that *Platycodon grandiflorum* root-derived saponins (PSs) exhibit antioxidant (Lee et al., 2004), anti-inflammatory (Choi et al., 2015; Kim et al., 2006a), hepatoprotective (Lee et al., 2008), anti-allergic (Han et al., 2009), and anti-obesity

Abbreviations: BUN, blood urea nitrogen; CK, creatinine kinase; ERK, extracellular-signal-regulated kinases; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinase; MHC, myosin heavy chain; MMP, matrix metalloproteinase; PS, platycodon grandiflorum derived saponin

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(Hwang et al., 2013) effects. There is the evidence for the inhibitory effects of PSs on NF-κB and MMPs signaling under inflammatory conditions (Choi et al., 2015; Zhang et al., 2017; Hwang et al., 2011). Therefore, we proposed a hypothesis that PS might exert protective effects on skeletal muscle damage by modulating NF-κB and MMPs. To test this hypothesis, we examined the effects of PS on eccentric exercise-induced muscle damage. Our results provide the first evidence that the administration of PS protects against eccentric exercise-induced muscle damage.

2. Materials and methods

2.1. Animals and PS extract

Eight-week-old male ICR mice were obtained from Daehan Biolink (Seoul, South Korea) and acclimatized to the experimental facility for 1 week. Mice were housed in a controlled environment (22–23 °C, 12/12 h light/dark cycle) in accordance with the Chungnam National University Animal Ethics Committee (CNU-0068). PS extract was prepared as previously described (Choi et al., 2014). Saponin were purified from *Platycodon grandiflorum*, and their composition were previously determined by high-performance liquid chromatography (Supplementary Fig. S1). PS-4 mg/kg were administrated via oral gavage once daily for 2 weeks. Experimental animals for PS administration were randomly divided into three groups to assess muscle damage protection as follows: REST: rested control group (n = 6), PBS: control group given PBS and subjected to eccentric treadmill exercise (n = 6), and PS-4: mice given 4 mg/kg PS for 2 weeks (n = 6). Four hours after performing eccentric treadmill exercise for 70 min, animals were sacrificed by cervical dislocation. The gastrocnemius muscle and blood were removed, immediately frozen in liquid nitrogen, and stored at −80 °C until use.

2.2. Induction of muscle damage

Eccentric exercise was performed on a treadmill 24 h after 4 mg/kg PS administration according to the eccentric exercise protocol (16 m/min for 70 min, −16 downhill). Mice underwent to start running at speed of 10 m/min with a downhill grade of −16 for 10 min. After 10 min, the speed was increased to 16 m/min at −16 downhill and done for 60 min (Lomonosova et al., 2014).

2.3. Measurement of damage markers in serum

The effect of PS supplementation on the levels of serum glucose, lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine kinase (CK), and C-related protein were evaluated 4 h after eccentric treadmill exercise for 70 min. Glucose, BUN, CK, and C-related protein levels in the serum were determined with an autoanalyzer (Toshiba TBA-40FR, Japan) on the same day.

2.4. Quantitative real-time polymerase chain reaction (RT-PCR)

Total RNA was isolated from untreated and PS-treated gastrocnemius muscles and cells using RNAiso-plus Reagent (TaKaRa Bio Inc. Shiga, Japan). After RNA isolation, complementary DNA (cDNA) was synthesized using a reverse transcription kit (Bio-Rad. USA). Accumulated PCR products were detected by monitoring increases in SYBR reporter dye fluorescence. The following primers were used: iNOS, Forward primer: 5′-CAG CTG GGC TGT ACA AAC CTT-3′, Reverse primer: 5′-CAT TGG AAG TGA AGC GGT TCG-3'. Caveolin3, Forward primer: 5′-GTT GTC TAC ACT GCT GGG TG-3′, Reverse primer: 5′-TAG CTC TTA ATG CAG GGC AC-3'. MG53, Forward primer: 5′-TCC TCA TGA AAT TCT GCC TGG TA-3′, Reverse primer: 5′-GGA TAT CTA GCC TTG CCG GT-3'. MMP-9, Forward primer: 5′-GCT GAA GCT CTG ATG TAC CC-3′, Reverse primer: 5′-TGT GGG AGT TCC ATA GAG GG-3'. MHC, Forward primer: 5′-CTC AGG CTT CAA GAT TTG GTG G-3′, Reverse primer: 5′-TTG TGC CTC TCT TCG GTC ATT-3'. TNF-α, Forward primer: 5′-ATA CGT CAG ACA TTC GGG AAG CAG TG-3′, Reverse primer: 5′-AAT AGT TGG TAT CCA GGG CTC TCC G-3'. IFN-γ, Forward primer: 5′-GCA CCA TCT TCA AGG GCA ATT TG-3′, Reverse primer: 5′-AGG AAG GAC AAG GAG ACC AAG G-3'. 18s, Forward primer: 5′-GTA ACC CGT TGA ACC CCA TT-3′, Reverse primer: 5′-GTA ACC CGT TGA ACC CCA TT-3'. The quantity of each transcript was calculated as described in the instrument manual and normalized to that of 18S, a house-keeping gene.

2.5. Western blot analysis

Following treatment, isolated gastrocnemius muscles were lysed in lysis buffer on ice for 30 min and centrifuged at 22,250 g for 20 min. Supernatants were collected and protein concentrations were measured using a protein assay kit (Intron Bio Inc. Korea). Aliquots of the lysates were boiled and electrophoresed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were transferred to polyvinylidene fluoride membranes, which were incubated with the appropriate primary antibodies. The membranes were then incubated with a secondary anti-mouse or anti-rabbit antibody. Finally, the protein bands were detected using an enhanced chemiluminescence detection kit (BIOFACT Inc. Korea). The integrated optical density of each protein band was calculated using ImageJ software. Values were normalized to the housekeeping gene α-tubulin or to the non-phosphorylated protein.

2.6. Histological tissue staining

Muscle tissue was collected and immediately fixed in 10% formalin after being weighed. Tissue was then embedded in paraffin and cut into 4-μm thick slices, stained with hematoxylin and eosin (H&E), and examined under a light microscope equipped with a CCD camera (BX-51, Olympus, Tokyo) by a clinical pathologist as previously described (Huang et al., 2012).

2.7. Statistical analysis

All data are expressed as the mean ± standard deviation of the mean. Differences among groups were analyzed by one-way analysis of variance with SPSS22 (IBM, Armonk, NY, USA). $P < .05$ was considered statistically significant.

3. Results

3.1. PS treatment protected against eccentric exercise-induced muscle damage

H&E staining was performed in the gastrocnemius muscle following 2 weeks of PS supplementation. The level of destroyed fiber in PBS group was prominent in the perimysium and endomysium at 4 h after eccentric exercise compared to the REST group. The PS-4 mg/kg supplementation group showed the least destroyed structure compared to the PBS group (Fig. 1).

3.2. Levels of muscle damage markers in the serum following eccentric exercise-induced muscle damage

The status of muscle damage at 4 h after eccentric treadmill exercise can be evaluated by examining important muscle damage indicators, including glucose, BUN, CK, creatinine, LDH, and C-related protein. Eccentric exercise caused muscle damage by increasing the serum LDH level, which peaked to 438.52 ± 41 U/L and decreased to 355.3 ± 52.7 U/L in the PS-4 mg/kg group. The serum CK level was significantly lower in the PS supplementation group compared with the